

WBC 2004 Abstracts

TECHNICAL SESSION I: Malt

Moderator: Scott Heisel

Scott Heisel received a B.S. degree in biochemistry and a B.S. degree in agronomy from the University of Wisconsin–Madison in 1982. In 1986, he received his M.S. degree in agronomy. Scott worked for several years at the USDA/ARS Barley and Malt Laboratory and has published several papers on characterizing various enzymes of germinated barley and the use of biochemical techniques to identify barley varieties. He joined the American Malting Barley Association, Inc. (AMBA), Milwaukee, WI, in April of 1987 and currently is the vice president and technical director. Scott is an active member of the American Society of Brewing Chemists, serving as chair of Local Section 4, chair of technical subcommittees, and national treasurer (2001–2003). As a member of MBAA, Scott has participated as a guest lecturer for the Malting and Brewing Short Course and spoke at several national meetings. He also is a member of the National Barley Improvement Association.

O-1

New Method for Malt Treatment by Subcritical Water

KOICHI NAKAHARA, Norihiko Kageyama, Koji Nagao, Takako Inui, and Nobuyuki Fukui

Process Development Department, Engineering & Process Development Division, Suntory Ltd., Yamazaki, Shimamoto-cho, Mishima-gun, Osaka, Japan

Papers have been published in the field of extraction, purification, and decomposition using supercritical fluid technology. In industry, methods of supercritical CO₂ have been already applied to several food production processes, e.g., removal of caffeine from coffee. Meanwhile, in the brewing industry, this method has been applied to the extraction process of alpha-acid and essential oils from hops. However, supercritical water technology has never been introduced to the brewing or malting industry. We have been developing industrial applications of supercritical and subcritical water for malt treatment. The malt tissue was hydrolyzed by water molecules and hydrogen ions at the near-critical temperature of water. By this method, several kinds of flavor and aromatic compounds were generated in a few minutes. The hydrothermal method resulted in an advantageous treatment to obtain the enhanced cooked flavors from the malt. This treatment gives a great advantage for the development of a new type beer.

Koichi Nakahara received a doctor's degree in agriculture from Kyushu University in Japan. Koichi graduated from Kyushu University in 1986 and graduated from graduate school of Kyushu University in 1988, majoring in applied microbiology. Since then, Koichi has been employed by Suntory Ltd. as a researcher. He has worked in the bioorganic chemistry laboratory and in the research area for natural products, Institute for Fundamental Research. Currently, Koichi is a chief chemist and general manager in the Process Development Department. Koichi works in the field of applied supercritical water technology.

O-2

Wort Amino Acid Composition of Different Barley Varieties and Effect on Nitrogen Assimilation

XIANG S. YIN, Gustavo H. Strasser, and William J. Ladish
Cargill Malt

The free amino acid composition of laboratory worts prepared from malt samples covering more than 20 varieties of malting barley from North America, South America, and Europe were studied. The patterns of the amino acid classes were examined across varieties. Significant differences were observed in the relative content of the Group D amino acid, proline, between the two-rowed and the six-rowed varieties. Ratio of other groups of amino acids was, therefore, affected accordingly. Results indicated that, within one variety, the percentage of each class of amino acids did not vary significantly due to malting process that was reflected by degree of modification and Kolback Index. At the same levels of soluble protein, the assimilable nitrogen level can vary by up to 10% depending on variety. The pattern of consumption of assimilable nitrogen during fermentation is also demonstrated using malt from different sources. Potential effects of

the amino acid composition on yeast performance and beer quality were investigated.

Xiang S. Yin is the technical manager for Cargill Malt, Americas, based at Prairie Malt Limited, Canada. He obtained his first degree in engineering in fermentation technology at Wuxi, China, and received his Ph.D. degree in 1986 from the Department of Brewing & Biological Sciences, Heriot-Watt University, Edinburgh. He carried out his postdoctoral research at the University of Edinburgh and then at the Grain Research Laboratory in Winnipeg. As the recipient of the 1990 Centenary Research Award of the Institute of Brewing, Xiang worked at the Brewing Research International, England, on beer flavor in the same year. He was an associate professor at the Wuxi Institute of Light Industry in China for 3 years before joining Prairie Malt as director of technical services in 1991. Xiang is the author or coauthor of more than 30 scientific and technical papers for international conventions and publications.

O-3

Choice of Enzyme Solutions Should Determine the Choice of Raw Materials and Process—Not Vice Versa

STEN AASTRUP, Noel Bautista, Elmar Janser, and Kurt Doerreich
Novozymes

Beer production has always been dependent on enzyme activity and their limitations. The current presentation summarizes how the enzyme content and enzyme activity of malt has determined the brewing regimes and the choice of raw materials. It is discussed to what extent malt analyses and malt specifications can secure raw material supply of uniform quality and, thus, a predictable and controllable brewing process. It is shown how scientists, barley breeders, maltsters, and enzyme producers have complied with the wishes from the brewers. 1) To extend their flexibility in choice of raw materials. 2) To increase extractability. 3) To secure rapid and trouble-free brewing. 4) To make special products. The main tools being 1) selection of new barley varieties with more accessible endosperm and much higher enzymes potential; 2) use of external enzymes with new or improved abilities compared with malt enzymes; 3) use of starter cultures for malting, resulting in increased enzyme activity in the malt; 4) introduction of new enzymes or enzymes with improved abilities in barley varieties using gene technology. Currently, the use of external enzymes gives the brewer the highest flexibility in the choice of raw materials, processes, and final products. Many breweries, however, do not benefit totally from the possibilities given by the much broader action frame of these enzymes. Many brewers just add the enzymes and keep the existing process conditions. This presentation outlines how choice of raw materials, raw material specifications, and processes can be significantly changed in favor of better processing and better beer quality (e.g., improved flavor stability) by choosing the right combinations of enzymes. The final part of the presentation will concentrate on future solutions. These shall be based on the increased desire for flexibility to meet the requirements from the customers for a higher diversity of products with constant, recognizable quality and high flavor stability. Here, the brewmasters have to "play on the whole piano". From classical "tunes" to completely new beer types. This means that the brewer will start with designing the beer and the beer quality and then simply ask for the raw materials and the enzymes that can do the job. The brewer shall be able to concentrate on the most important aspect—beer quality—and let the enzymes take care of productivity and economy. This will put an increased pressure on the enzyme producers (maltsters and producers of external enzymes) to make more specific solution the specific requirements.

Sten Aastrup received his M.S. degree in biology from the University of Copenhagen in 1979. Since then, he has worked in the brewing industry for 10 years as a scientist and senior scientist at the Carlsberg Research Center; 5 years as head of the Carlsberg Malt House; and 9 years as senior consultant at Alfred Joergensen Laboratory. In 2004, Sten began employment with Novozymes as technical service manager.

O-4

NIR Spectroscopy for Single-Kernel Analysis—A Novel Tool for the Evaluation of Homogeneity in Barley and Malt

FRANK RATH (1) and Frank Nitzsche (2)

(1) VLB Berlin, Germany; (2) König Brauerei, Germany

An essential precondition for the rational and economic production of top quality malt and beer is the consistent high quality of the raw materials

used. Inhomogeneous barley and malt can be a key cause of technological and qualitative problems, which make the industrial processing more difficult and more expensive, as well as reducing the quality of the final product. Paying more attention to the homogeneity of the barley and malt when evaluating their quality is thus an essential precondition for a more precise prediction of their performance in the malting and brewing processes and can lead to a further noticeable improvement in the quality of the raw materials. An innovative method to measure the homogeneity of barley and malt samples is presented that utilizes the special advantages of near-infrared technology based on a diode-array spectrophotometer for the rapid analysis of a very large number of single kernels. For the homogeneity analysis, a special measuring device was developed that uses glass-fiber optics to allow the intake and evaluation of spectral data from up to 10 different points per kernel. Several detectors are arranged in a staggered formation in order to minimize such interfering factors as the kernel shape, size, and orientation. The single kernels are fed in a continuous stream by an automatic mechanism. Unmodified and partially modified regions of the malt endosperm were identified and quantified on the basis of their NIR spectral data. The spectral information was evaluated with the help of analytical reference data obtained from homogeneity analysis using the calcofluor staining of the endosperm (Carlsberg Method). With the help of multivariate statistical algorithms, calibration methods to measure the homogeneity of the malt modification were calculated and validated. The model allows the homogeneity of endosperm modification of an unknown sample to be predicted purely on the basis of its spectral data. Further calibration models were developed for other important grain constituents—protein, moisture, and beta-glucan. The homogeneity analysis with NIR technology is a nondestructive method requiring no sample preparation and allowing a high analysis rate. It thus fulfills the necessary conditions to be able to analyze large samples as a basis for a high statistical security and good result reproducibility. The NIR-based single-kernel analysis thus overcomes the disadvantages and weaknesses of conventional homogeneity methods that limited their wider application. In the future, calibration models should be developed for other interesting kernel constituents and for other grain crops.

Dr. Frank Rath was born in 1957. Frank studied agricultural science at the Rheinische Friedrich-Wilhelms-University of Bonn (1980–1986) and received a Ph.D. degree in 1993. Frank has been a scientific collaborator at the Research Department/Plant Production and Physiology, Weissheimer Malzfabrik, Andernach (1986); a scientific collaborator at the Research Institute of Raw-Materials within the Research and Teaching Institute of Brewing in Berlin (VLB) (1986–1990); head of the Research Department/Plant Production and Physiology, Weissheimer Malzfabrik, Andernach (1990–1998); and head of the Research Institute of Raw-Materials within the Research and Teaching Institute of Brewing in Berlin (VLB) (1999–present).

O-5 **Lipid Oxidation During Mashing and Its Impact on Beer Quality—Recent Progress**

HISAO KURODA (1), Naohiko Hirota (2), Hirotaka Kaneda (1), Naoyuki Kobayashi (1), Kazuyoshi Takeda (3), Kazutoshi Ito (2), and Masachika Takashio (1)

(1) Frontier Laboratories of Value Creation, Sapporo Breweries Ltd.; (2) Bioresources Research and Development Laboratories, Sapporo Breweries Ltd.; (3) Barley Germplasm Center, Research Institute for Bioresources, Okayama University

One of the most important issues in modern brewing is how to produce flavor- and foam-stable beer. We found that trihydroxyoctadecenoic acid (THOD), one of the products made by lipid oxidation during mashing, deteriorates both beer foam and the smoothness of beer. Lipid oxidation during mashing also results in the production of *trans*-2-nonenal (T2N) or its precursors, which are thought to be the major contributors to the stale flavor that arises during the storage of beer. We have focused on biochemical analyses of the enzymes involved in lipid oxidation during mashing and attempted to clarify how THOD and T2N are produced during mashing. We have already shown that linoleic acid hydroperoxide, the oxidative product of linoleic acid derived from malt, is produced by malt lipoxygenase; however, the pathways leading to THOD or T2N were unknown. The aim of the present study was to clarify these pathways. First, using recombinant protein technology, we proved that THOD was produced from the sequential oxidation of linoleic acid by malt lipoxygenase-1 and peroxygenase. Second, we discovered the presence of

9-fatty acid hydroperoxide lyase in malt and showed that it enzymatically cleaved 9-hydroperoxide of linoleic acid into T2N during mashing. Interestingly, both peroxygenase and 9-fatty acid hydroperoxide lyase showed higher heat stability than did lipoxygenase, so that the activities of these enzymes would survive after the inactivation of lipoxygenase and produce THOD and T2N during mashing. This indicates the importance of not only lipoxygenase but also peroxygenase and 9-fatty acid hydroperoxide lyase regarding the mashing methods, the processing methods of malt production, and the selection of types of malts or barley cultivars. In addition, because THOD and T2N are produced by this enzyme cascade during mashing, we predicted that if we efficiently block the first reaction, which produces 9-linoleic acid hydroperoxide by lipoxygenase-1, this would result in the reduction of THOD and T2N in beer, thus enabling the production of foam- and flavor-stable beer. Excitingly, the theory was recently proved by the result of brewing trials using the LOX-less barley line, which completely lacks authentic lipoxygenase-1 protein.

Hisao Kuroda received an M.Sc. degree from the Department of Biology of the Graduate School of Nagoya University in 1989. He then joined the Plant Bioengineering Research Laboratories, Sapporo Breweries Ltd. as a research scientist and worked on the biotechnology of malting barley. From 1998 to 2002, he worked on the malt enzymes related to lipid oxidation as a lead biochemist at Brewing Research Laboratories, Sapporo Breweries Ltd. Currently, Hisao studies the barley enzymes involved in lipid metabolism through a genomics and proteomics approach at Advanced Technology, Frontier Laboratories of Value Creation, Sapporo Breweries Ltd.

O-6 **The First PCR Marker for Breeding of High-Quality Winter Malting Barley—A Novel Selection Tool Against Beta-Amylase-Weak Genotypes**

MICHAEL VOETZ and Frank Rath
VLB Berlin, Germany

The amylolytic enzyme beta-amylase plays a key role in the production of fermentable sugars during the mashing process. In contrast to spring barley, different genotype-dependent thermostabilities of the enzyme could not be observed in winter barley. Nevertheless, extensive analyses of winter barley genotypes (cultivars and breeders' lines) showed that the level of beta-amylase activity varies by up to a factor of 3 to 4 when assaying genotypes grown under similar environmental conditions. After comparative enzyme analyses of numerous genotypes harvested at different locations, we have clear evidence that the highest achievable level of beta-amylase activity is a heritable trait in winter barley. To elucidate the molecular basis of this phenomenon, we first examined whether differential activation of the enzyme is responsible for the dramatic differences in beta-amylase activity. The beta-amylase is synthesized during grain development and needs to be converted into an active form during germination. The enzymatic reduction of disulfide bonds between the enzyme and other grain proteins and an endoproteolytic removal of C-terminal peptides are discussed as 'activating processes'. Since we could ascertain the same ranking of genotypes with respect to beta-amylase activity in barley and malt, the C-terminal processing of the protein could be excluded as a decisive reaction. We obtained similar results when omitting the reducing agent in the enzyme assay, thus proving that the differences in beta-amylase activity cannot be due to differences in enzyme activation. To find out if differential gene expression of the beta-amylase gene is crucial to the activity levels of the enzyme, we characterized the promoter sequences from beta-amylase genes isolated from genotypes with extreme high and low enzyme activity, respectively. The exchange of a single nucleotide within an I-Box-like promoter element could be detected in the low-activity genotypes. Based on this polymorphism, we designed selective PCR primers. Subsequent PCR analyses and enzyme assays of more than 100 varieties and breeders' lines revealed that, without exception, the winter barley genotypes with low beta-amylase activity carry the mutation in the promoter sequence indicated by a positive PCR result. The data presented are the basis for a novel and highly focused marker-assisted selection aimed at high DP values by deletion of beta-amylase-weak genotypes. It is now possible, for the first time, to select in early breeding generations without micromalting, thus achieving a substantial shortening of time spent on the breeding of optimized winter barley varieties.

Michael Voetz, born in 1964, received a diploma in biology from the University of Cologne in 1991. He earned a Ph.D. degree in plant molecular biology from the University of Cologne/Max-Planck-Institute for Breeding Research in 1995. From 1995 to 2000, he was scientific collaborator at the Research Department of the Weissheimer Malzfabrik in Andernach, working in the field of barley biotechnology. Since 2000, he has been head of the biotechnology/PCR laboratory at the Research Institute for Raw-Materials within VLB in Berlin.

TECHNICAL SESSION II: Beer Analysis

Moderator: Cindy-Lou Dull

Cindy-Lou Dull received a B.S. degree in dairy science from the University of Vermont and earned an M.S. degree in food science from Cornell University. She began her career in the development of rapid methods for the food and forensics industries before finding her niche in the brewing industry. In 1992, she joined corporate research and development at Anheuser-Busch, Inc., St. Louis, MO, as a microbiologist with her efforts directed toward aseptic brewing issues and rapid methods evaluation. A member of the Technical Center since 1994, she has worked in various capacities, most recently as a scientist in the Analytical Services group and as liaison to corporate brewing customers. She has enjoyed being an active member of ASBC since 1994, having participated in several subcommittees, chairing the subcommittee for CLEN Medium for the Detection of Wild Yeast, and serving on the Technical Committee, currently as chair. Cindy-Lou is well known by her friends and colleagues for her daily dose of inspirational quotes via e-mail, "Food for Positive Thought".

O-7

A New Brewing Science Study in the 21st Century Fused with Brain Science—Measurement of Human Brain Activity Evoked by Stimulation of Beer Bitterness Using Magnetoencephalography
HIROTAKA KANEDA (1,2), Naomi Goto (2), Tatsu Kobayakawa (2), Masachika Takashio (1), and Sachiko Saito (2)
(1) Sapporo Breweries Ltd.; (2) National Institute of Advanced Industrial Science and Technology

One of the ultimate questions for brewing scientists may be where and how human beings identify the beer tastes and odors and evaluate their pleasantness. Bitterness is an important factor for beer pleasantness, although it is generally an unpleasant factor for foods and beverages. It is quite interesting for brewing scientists as to how the brain functions to make human beings feel pleasure when tasting beer. To answer this question, the fusion of brewing science studies with brain science and psychological studies will be indispensable and will provide new values in the creation of marketing strategy. We tried to noninvasively detect the human brain activity evoked by beer bitterness using magnetoencephalography (MEG). The measurement principle of the MEG is as follows. When information is being processed (neural activities occur), small currents flow in the brain, producing small magnetic fields. The MEG measures the magnetic field outside the scalp with a superconducting quantum interference device magnetometer. An equivalent current dipole (ECD) can be mathematically calculated from the distribution of the magnetic fields on the scalp. The taste delivery system has been developed. Deionized water and beer were presented to the tongue through a Teflon tube in the system by compressed air. The duration of each stimulus was 600 ms in one trial (total trials: 40) and the interstimulus interval was about 30 s, during which the tongue was continuously rinsed with deionized water. The MEG measured the changes in the magnetic fields on the scalp based on the brain activation in the recognition of the beer tastes. Although the magnetic fields did not significantly change during the stimulation by water and beer, the increase in the magnetic fields was observed for the stimulus of the beer with the addition of isohumulones, indicating the specific brain activation for isohumulones. The ECDs for the stimulation with beer enriched with isohumulones, placed on a subject's three-dimensional magnetic resonance imaging, were located at the transition between the parietal operculum and the insular cortex (area G) with a latency at 326.7 ± 6.115.5 ms. It has been reported that area G is the primary gustatory area in the human brain. The results indicated that the brain activity stimulated by beer bitterness could be detected. Our studies will be the first step in the fusion of brewing science with brain science, leading to the clarification of the evaluation mechanisms for the pleasantness of beer tastes and to supporting the sensory evaluation studies of beer tastes.

Hirotaka Kaneda is a general manager of advanced technology at Frontier Laboratories of Value Creation of Sapporo Breweries Ltd. He graduated from Kyushu University in 1984 with a M.S. degree in food hygienic chemistry and then joined Sapporo Breweries Ltd. He has investigated beer stability and received a Ph.D. degree in food science from Nagoya University in 1994. He has studied the human brain function during gustation and olfaction as a guest researcher at the National Institute of Bioscience and Human Technology from 1996 to 2001. He received the Eric Kneen Memorial Award from the ASBC in 1995 and the Technical Award from the Agricultural Chemical Society of Japan in 2000. He is currently a member of the editorial board for the American Society of Brewing Chemists (2001–present).

O-8

Stable Isotope Dilution Assay of Methanethiol and Dimethyl Trisulfide in Beer Using a Purge and Trap Method
SACHIO IINUMA, Emiko Koremura, Tetsuji Yasui, Shuso Sakuma, and Motoo Ohkochi
Kirin Brewery Co., Ltd.

Sulfur compounds generally exhibit intense aroma properties due to their low olfactive thresholds. Methanethiol and its polysulfide, dimethyl trisulfide (DMTS), most often occurred at levels above their olfactive threshold in aged beer with nauseous sulfur-linked smells. As methanethiol is very oxidizable and chemically reactive, measurements of such compounds in their low threshold levels were considered very difficult. So stable isotope dilution assays (SIDA) of both methanethiol and DMTS in beer using purge and trap sample concentration techniques were developed. These sulfur compounds were measured by purging them onto Tenax adsorbent and then injecting the volatiles onto a GC column using thermal desorption and cryogenic focusing. Sulfur compounds eluting from the column were quantified by GC/MS. Using this method, we determined the levels of methanethiol and DMTS in various brands of Japanese commercial beer and happou-shu from different breweries. As a result, it was found out that the levels of these sulfur compounds differ both between the brands and within the same brand from different breweries. We consider that our SIDA methods will be a powerful tool to regulate these sulfur compounds in breweries. This paper will also discuss the influence of pH, headspace air, sulfur dioxide, ferrate, and ethylenediaminetetraacetic acid (EDTA) in beer and happou-shu during aging.

Sachio Iinuma graduated from Tsukuba University in 1992 with a master's degree in agricultural chemistry and then joined Kirin Brewery Company Limited. Sachio worked in the Research And Development Department from 1992 to 2002. Since 2002, Sachio has worked in the Technology Development Department, Research Laboratories for Brewing.

O-9

Development of a Multiresidue Analysis Method of Agrochemicals Using Liquid Chromatography/Tandem Mass Spectrometry
MASAYUKI OMOTE, Kouichi Harayama, Tomoko Sasaki, Naoki Mochizuki, and Hiroshi Yamashita
Asahi Breweries, Ltd.

In Japan, it was decided to enforce the positive list system for regulating pesticide residues by a newly revised legislation governing sanitation, and the first draft on proposed provisional limits has been published to regulate more than 500 agricultural chemicals. After the legislation was introduced, the scope of application has expanded to include not only raw materials but also processed food. Therefore, manufacturers are obliged to confirm their compliance with the legislation by self-imposed inspection. This situation led us to develop a rapid method for simultaneously analyzing multiresidue chemicals with simple pretreatment and high sensitivity. In this study, we attempted to develop a simple method for analyzing multiresidue chemicals using liquid chromatography/tandem mass spectrometry (LC/MS/MS). We were able to determine them in pale malt and beer products with a simple pretreatment process that includes liquid-liquid extraction followed by solid-phase column purification. In the case of hops, coextracted matrix components, for instance pigments and hop oil, may strongly influence the ionization efficiency of target analytes, and these matrix effects should be minimized. We reduced coextracted matrix components using gel permeation chromatography

(GPC). As a result, the simultaneous analysis of over 250 chemicals was achieved with high sensitivity and the detection limits were found to be 0.01 mg/kg (ppm) or below. We analyzed raw materials and beer products and confirmed that all of them complied with the proposed provisional limits published in the first draft. These results indicate that our newly developed method has potentially extensive applications to quality assurance.

Masayuki Omote is an analyst at Analytical Technology Laboratory, Asahi Breweries Ltd. He graduated from the Graduate School of Pharmaceutical Science of Kyoto University and joined Asahi Breweries Ltd. in 2000. He has been engaged in the research and development of analytical technology since 2000.

O-10

Wort Turbidity—Comparison of Different Measuring Principles

ARNOLD ROGNER and Ralf ISENBERG
Sigrist-Photometer AG, Switzerland

The turbidity or solids content in wort after the lauter tun has a significant influence on the quality of the brewing process and also the final product. The target clearly is to have a turbidity as low as possible. The MEBAK directive for on-line turbidity measurement in wort specifies to use forward-scattered light. The reason is to have a good relation to the suspended solids concentration. On the other hand, other measuring principles, such as 90-scattered light, back-scattered light, and absorption measurement, are used by various manufacturers. In 2003, we had done a couple of investigations with some breweries and a brewhouse manufacturer on these different technologies to learn about the differences and the interpretation of the results. Forward-scattered light indeed gives the best correlation with the suspended solids. Since this is the parameter that should be kept at a low level for improved processing during wort boiling and fermentation, it is the most relevant one. However, back-scattered light gives very similar results, with a more easy technology. 90-scattered light does not help to improve the lautering process but can help to identify bad malt quality. Therefore, it cannot be more than supplemental information. Finally, absorption measurement, although under widespread use, gives unrealistic high values at the end of the process when cutters are applied. The paper compares the different technologies and demonstrates and explains the differences in the results. Generally, the preferred technology depends, in fact, on what you will learn from the wort turbidity measurement and also from the state-of-the-art of the brewhouse technology.

Arnd Rogner received his Ph.D. degree in physics in 1989 from Karlsruhe University in Germany. After working several years as development engineer, development manager, and product manager in the field of fiber optics and optical sensors, he joined Sigrist, a leading manufacturer of brewery turbidimeters, in 1997. As marketing manager, he helped to define and launch the actual Sigrist product line of turbidimeters, color, and dust monitors. Since 2000, he has been responsible for the worldwide sales activities of Sigrist.

O-11

Toward Improved Fermentation Consistency Using Multivariate Statistics

Jeff Hodgson (1), Hilary Jones (1), Jim Robertson (2), Greg McFarlane (2), Steve Bland (2), David Hopper (2), Kate Kemsley (3), Marianne Defernez (3), and BEHNAM TAIDI (1)

(1) Scottish Courage Ltd. Technical Centre, Edinburgh, U.K.; (2) Charles Wells, Bedford, U.K.; (3) Institute of Food Research, Colney, Norwich, U.K.

Beer production can be an inherently variable process due to the involvement of so many biological processes during its production and the complex nature of the raw materials involved. Central to beer making are wort production and fermentation. Wort production can be automated and controlled through accurate real-time data management systems. The fermentation part of the process, however, offers fewer opportunities for such a level of control and human judgement often plays an important role in ensuring each batch of product meets all specifications. While considerable research has been undertaken on analyzing and controlling pharmaceutical fermentations, this expertise is not directly transferable to brewing due to inherent differences from pharmaceutical conditions. There are important commercial benefits to be gained from an

improvement in fermentation consistency. The potential business benefits can be divided into two broad areas, namely, management of product supply to customers and brewery capacity increase. An initial attempt to measure the consistency of fermentations at Scottish Courage Ltd. led to the development of centile lines for the measurement of the shape of fermentation curves. This, in turn, led to a larger project involving a consortium consisting of Charles Wells Brewery, the Institute of Food Research, and the U.K. Government's Department of Environment, Food and Rural Affairs. The objectives of this project are as follows. • To understand and characterize natural variability in fermentation performance, as measured by a range of process variates and beer qualities. • To develop generic predictive protocols that will provide early indications of deviations from nominal acceptable fermentation behavior. • To develop models that can predict the end time-point of fermentations, using multivariate data collected in the period immediately after pitching (24–48 h). The approach adopted is very much based on statistical analysis and model construction rather than a mechanistic approach. This presentation will discuss the early challenges encountered and the progress made during the first year of this project.

Behnam Taidi (B.Sc., Ph.D., AMIBREW) is the research and development manager for Scottish Courage Ltd. He is in charge of progressing the strategic research program by initiating and managing process innovation projects. Behnam has more than 10 years of experience in brewing research and, although his expertise is in the area of fermentation and yeast, he manages projects in many diverse areas such as novel raw material usage, yeast management, fermentation control, by-product utilization, rapid microbiology, and beer quality. Behnam serves on the Scottish Section IGB committee and regularly attends scientific and brewing conferences, where he presents aspects of his research.

O-12

Multivariate Analysis of Routine Beer Analysis Methods

KARL J. SIEBERT
Cornell University, Geneva, NY

Brewing companies develop analytical methods and apply as routine those that are found to contribute useful information, often to solve a particular problem. In most cases, the entire set of methods is never examined collectively to see the extent to which some of them may be redundant and to consider the possibility that a well-chosen subset of procedures might provide as much information as all of them collectively. This has the potential of saving both time and money at little or no cost in loss of information. A study of the collective information content of 47 analytical observations applied to at least six samples each of 10 beer brands was carried out. Principal components analysis (PCA) was used to determine the number of fundamental properties represented in the data set and, thereby, to estimate the degree of redundancy. When samples of one of the brands were included, they tended to dominate the PCA because they were so different from the other beers. The data set was then examined with these samples removed. It appears that the number of significant principal components (PCs) is on the order of seven or eight, depending on the criterion used. This indicates that the 47 measurements together only contained information on seven to eight fundamental properties, and there was considerable redundancy. The first two PCs contained information that was sufficient to almost completely separate samples of the 10 brands. Eight PCs were Varimax rotated and the factor loadings were examined to determine the influence of the measurements on the PCs and to estimate the factors that were, to some extent, redundant. It was possible to identify 14 measurements from the 47 used that captured most of this information and retained much of the ability to separate brands. Hierarchical cluster analysis was applied to the transposed data matrix and to a correlation matrix of the measurements to obtain two additional views of the relationships between the measurements. The results from the correlations were intuitively more satisfying and showed both known and some unexpected relationships between measurements. Several pattern recognition procedures were applied to attempt classification of the samples by brand. This was quite successful with linear discriminant analysis (LDA), K-nearest neighbor analysis (KNN) and soft independent modeling of class analogy (SIMCA). Reduction of the number of measurements used by selecting those of greatest classification utility improved the classification ability of LDA and SIMCA; this produced the best results with 11 and 17 measurements, respectively.

Karl Siebert received a Ph.D. degree in biochemistry from Penn State in 1970. He joined the Stroh Brewery Company in Detroit, where he spent 18 years and held positions from research associate to director of research. In 1990, Dr. Siebert joined Cornell University as professor of biochemistry in the Department of Food Science and Technology. He served 5 years as department chairman and now has a predominantly research appointment. Dr. Siebert served on ASBC technical subcommittees and was a member and chairman of the Technical Committee. He is serving his second stint on the Journal of the ASBC editorial board (1980–1992; 1996–present). He is active as a consultant in the beverage industry.

TECHNICAL SESSION III: Filtration

Moderator: Frederik Havel

Fred Havel received his B.Sc. degree in crop science in 1981 from the University of Guelph's OAC in Guelph, Ontario. He joined the brewing industry as an apprentice maltster with Canada Malting Co. on Bathurst Street in Toronto in 1983. Over the next two decades, he has held a number of positions with Carling O'Keefe, Molson, SUN Brewing, Carlsberg, and as a private consultant. Fred has worked in Canada, the Caribbean, China, Africa, and throughout Europe and the ex-Soviet block. Fred is currently a development brewer in Molson's Global Quality and Innovation Department. Fred is a member of MBAA District Eastern Canada.

O-13

Cost and Quality Comparison Between DE and Crossflow Filtration for Beer Clarification in Industrial Scale

HANS DENNIGER (1) and Reiner Gaub (2)

(1) Westfalia Separator AG; (2) Pall Food & Beverage

This paper compares the individual consumption data with related costs between a traditional kieselguhr filter (plate-frame) and a modern high-efficiency centrifuge/crossflow system (PROFI, Westfalia/Pall). Both technologies have been installed in parallel in a German midsize brewery operating the same beers under identical conditions. During a period of 6 months, all data were measured and recorded by an independent institute (Tech. University of Vienna). Results are as follows. • Investment comparable with crossflow and DE filterline. • Beer and extract losses significantly lower with PROFI system. • Crossflow eliminates filtermedia handling completely. • Water consumption significantly lower with crossflow. • Manpower is significantly lower with crossflow. • Energy consumption for crossflow lower than for DE with poor filterability of beer, with good filterability vice versa. • Downtime: CMF is continuous operation, DE is batch operation; this results in smaller sizing for CMF systems. • Flexibility: CMF allows for an easy change between brands within minutes without mixing phases. • Total costs: CMF is comparable to DE filtration, especially with poor filterability beers. • Quality: CMF is, in all aspects, comparable to DE but with better microbiological results, more constant haze, and significant lower oxygen intake. The paper gives detailed facts and figures for each criteria. Based on these results, the brewery has stopped using DE filtration and switched 100% to a PROFI system for all brands.

Hans Denniger was born in 1957, is married, and has two children. From 1972 to 1975, Hans held an apprenticeship as a brewer at the Iserlohner Brauerei, which is part of Brau & Brunnen, one of the German brewery groups. From 1975 to 1978, Hans practiced as a brewer in all production areas at the Vormann Brauerei. From 1978 to 1979, Hans studied at the VLB (Versuchs & Lehranstalt für Brauerei in Berlin) and received a degree as Braumeister VLB (master brewer VLB). From 1979 to 1988, Hans was a chemical/technical assistant and brewmaster of the Institut für chemisch-technische Analyse der VLB, Berlin, under Prof. Eckhard Krüger. Hans was responsible for the research and development of analytical procedures and analyses, as well responsible for the R&D brewery plant at the institute. From 1988 to 1990, Hans was involved in the engineering, commissioning, and running of microbreweries in Germany. Since 1990, Hans has been project manager, beer, at Westfalia Separator AG in Oelde. Hans is responsible for the development of new processes with centrifuges in breweries, especially for the development of a process on DE-free filtration (SWS = Seitz-Westfalia system or PROFIL) and continuous stabilization. Hans' hobbies include home brewing, sailing, and fishing.

O-14

The Filterability of Wort and Beer

DR. STEFAN KREISZ, Klaus Hartmann, and Prof. Werner Back
Institute for Brewing Technology I, Freising, Bavaria, Germany

This paper is the summary of two doctoral thesis about the filterability of wort and beer partly already published at the EBC Congresses in Cannes (1999), Budapest (2001), and Dublin (2003). It will describe the influence of the main sources of polysaccharides and proteins (malt, yeast, and bacteria) on the filterability (pressure increase at the filter inlet and turbidity at the filter outlet) of wort and beer. Analytical Methods: All malt, wort, and beer analysis were executed according to the Analytica EBC. Five new methods were invented: a method to predict the filterability of beer by analyzing the wort (published in Cannes); a method to identify polysaccharides after filtration by releasing them from kieselguhr (published in Budapest), a new method to estimate the risk of beta-glucan gel formation by intensive shearing of wort and beer (not yet published), a step control to identify the process step that causes the problems (partly published at WBC 2000), and a method to identify haze particles in filtered beer by use of specific enzymes in combination with haze measurement and staining methods. Results: The risk of beta-glucan gel formation was measured in 144 malt samples (six varieties, eight proveniences, three different malting regimes) and compared with three cytolytic parameters (beta-glucan content, viscosity, friability). The results show coherence between these parameters and the risk of beta-glucan gel formation. The influence of the three malt polysaccharides (alpha- and beta-glucan and pentosan) on the viscosity of wort was measured depending on the cytolytic modification of the malt. Six brews with different modified malts were executed twice: one time under standard conditions and one time by degrading the cell wall polysaccharides using heat-stable enzymes. The differences in lauter performance and filtration time were measured to demonstrate the influence of the cell wall polysaccharides on these production steps. The step control was realized in five different breweries, three of them having problems with poor filterability. The origin of the problems was identified and the filterability improved. Raw materials, including the yeast, can cause turbidity up to 2 EBC, which cannot be eliminated by a standard filtration. The origin of such haze particles can be detected with specific enzymes in combination with haze measurement and staining methods. The knowledge of the substances leads to technological solutions to minimize haze problems.

Stefan Kreiszi studied brewing and beverage technology at the Technische Universität München-Weihenstephan, Germany (1991–1997). He graduated as an engineer in 1997. From 1997 until 2002, he completed his doctoral thesis at the Institute for Brewing Technology I, concerning the filterability of wort and beer. From 2000 until 2002, he worked as a scientific employee and assistant at the malt laboratory at the Institute for Brewing Technology I. From 2002 until the present, he is head of the malt laboratory. His main research interests are barley, wheat, and malt and he also works as a consultant for malteries and breweries.

O-15

Practical Experiences with Membrane Filtration for the Clarification of Beer on an Industrial Scale

T. REINOU NOORDMAN (1), Marcel van der Noordt (1), Arie F. C. Hardenbol (1), Coen J. Peet (1), Lute Broens (2), and Andre Mepschen (2)
(1) Heineken Technical Services, Zoeterwoude, The Netherlands; (2) Norit Process Technology, Enschede, The Netherlands

At the WBC 2000 in Orlando, Heineken Technical Services and Norit Membrane Technology reported on a new cross flow membrane filtration process as an alternative to kieselguhr filtration. This membrane filtration process eliminates the use of kieselguhr and its associated problems, such as the disposal of spent kieselguhr waste and the workers' safety issues of the possible inhalation of kieselguhr dust. Since the WBC 2000, the commercial-scale membrane filtration units have been installed in breweries. These commercial-scale units are successfully clarifying beer on a daily basis and have proven to be adaptable to a wide variety of beers, including beers with very high yeast concentrations. Depending on the beer type, membrane filtration maintains high filtration fluxes (108 L m⁻² h⁻¹) at filter run lengths ranging from 7 to 20 h between membrane regeneration. The costs of commercial-scale membrane filtration for bright beer are currently approximately equal to those for kieselguhr filtration. The costs, product quality, operational aspects, and filterability of commercial-scale membrane filtration will be described for various types

of beer. Membrane filtration is successful for the clarification of beer due to a new oxidative agent for regeneration of the membranes. This oxidative agent regenerates clogged membranes in about 2 h and the membrane flux rates are maintained for more than 100 regenerations. We believe that membrane filtration is a viable commercial replacement for kieselguhr filtration.

Reinoud Noordman graduated as a chemical engineer in 1991 from the University of Groningen, The Netherlands. From 1992 to 1998, he was a Ph.D. candidate and carried out research work at the University of Groningen on various membrane filtration projects (desalination, wastewater treatment, and modelling) and product development (improvement of the shelf life of sweet products). In April 2000, he received a Ph.D. degree in the field of membrane filtration. Since January 1999, he has worked for Heineken Technical Services as a senior scientist and is involved in the development of new separation processes.

O-16

Crossflow Filtration of Beer—A True Alternative to Diatomaceous Earth Filtration

ALEXANDER MODROK, Dr. Bernhard Diel, Michael Rodenberg, and Dirk Weber
Sartorius AG, Goettingen, Germany

At breweries, crossflow filtration technology can replace diatomaceous earth filters and fine filters, such as sheet filters, disc filters, and trap filters. The subsequent use of sterilizing-grade membrane filter cartridges additionally eliminates the need of flash pasteurization. The combination of crossflow technology and membrane filter cartridges creates a new filtration system for breweries. Breweries who use this new concept receive a completely cold-filtered or nonpasteurized beer of the highest quality. Compared with conventional DE filtration, crossflow filtration of beer offers numerous advantages. The key aspect is that the use of kieselguhr or diatomaceous earth (DE) is no longer necessary. The fact that DE is a limited resource will have a negative impact on its quality and price in the future. Moreover, the disposal of DE generates additional costs. The health risks for the user associated with DE that contains cristobalite should not be underestimated as well. Fully automated crossflow systems are less labor-intensive to operate than are diatomaceous earth filters. In conjunction with lower water consumption and a lower product loss, process costs can be reduced. The use of crossflow technology does not have any negative influence on the quality of the beer, whereas yeasts and beer-spoiling organisms are reliably removed. In addition, crossflow filtration has a positive effect on the service life of downstream sterilizing-grade filter cartridges. Due to this fact, filtration costs can be reduced even more. This is a great additional cost advantage for breweries using membrane filters for the cold sterilization of beer. Further advantages of crossflow technology can be found in its excellent scalability. With these systems, scaling up or down is exceptionally easy. Sartocon filter cassettes are at the heart of every Sartflow filtration system. They feature optimized hydrodynamic properties, in which consistent crossflow rates across the membrane and short flow distances inside the membrane cassette modules are guaranteed. The equally optimized pressure ratios during filtration and cleaning allow high flow rates and efficient cleaning. The crossflow systems contains a new PESU membrane specially developed for beer filtration with an extremely low protein adsorption. The use of narrow channel and flat membrane modules with optimal hydrodynamic properties and an optimized process results in high flux rates of 70–100 L/m²/h and a very low energy consumption. In consideration of these facts, crossflow filtration of beer offers a true alternative to conventional DE filtration with comparable cost.

Alexander Modrok became a brewer and maltster in 1977 and then worked in several German breweries, including Beck's in Bremen. In 1982, he became a brewmaster and maltmaster after he visited the Brewmaster College in Ulm, Germany. He joined Sartorius in the position of technical support, and in 1987, he was promoted to the F&B market manager Europe. In 1995, he moved to Japan in the position of business united director F&B Asia Pacific. After returning to Germany in 1999, he was promoted to the position of head of global market management brewing industry within the Sartorius group. Here, he supported the development of new crossflow technologies for the brewing industry. Alexander is married to his wife Carmen, and they have one 18-year-old

daughter. They are living near the well-known university city of Goettingen in Germany.

O-17

Beer Stabilization Technology, Clearly a Matter of Choice!

MUSTAFA REHMANJI, Chandra Gopal, and Andrew Mola
International Specialty Products

Stabilization is an important stage in the production of beer, in which an attractive appearance and flavor are considered key quality determinants. The methods adopted to achieve good colloidal stability have changed over time. Advances in brewing technology, cost effectiveness of the technology employed, avoiding products that may leave residue in the final beer or detract from beer quality, and safe product handling, in terms of generation of dust and package size, are some of the key considerations in the selection of a suitable stabilizer/recipe. The history of beer stabilization is reviewed in this presentation to understand how the raw materials, technology adopted, and optimization of the brewing process have reached their current state and have impacted the final quality of the beer produced. Drivers for improvement in beer stability include the following: • Increased competition from imports. • Expanded distribution areas: beer traveling farther from the brewery. • Consumer expectations for better quality and product consistency. • Need to reduce costs from returns of beer. While current procedures usually concentrate on addition of the stabilizer after fermentation—e.g., on transfer to maturation or at filtration—little has recently been reported on determining efficacy of stabilization earlier in the brewing process. A procedure for upstream clarification/stabilization of wort in the brewkettle will be discussed. This should simplify the downstream stabilization and processing before filtration and reduce cost. This could be adopted in developing markets to achieve good colloidal stability without capital investment in specialized equipment. Elsewhere, it could provide an additional mechanism to chillproof 'difficult' beers in challenging environments. The possible future developments of stabilization technology are considered in light of past experience and likely future drivers.

Mustafa Rehmanji has more than 20 years of experience in the malting and brewing industry. He is section manager, beverage products, research and development with International Specialty Products based at the ISP Technical Center in Wayne, NJ, U.S.A. His current interest is in the area of beer stabilization and technical service for commercial treatment of beverages. Mustafa started his brewing career with Kenya Breweries. Later, he moved to Canada and was director of technical service with Prairie Malt Limited. Mustafa holds a B.Sc. degree in chemistry, a business degree, and a diploma in brewing technology. He is an active member of the ASBC and MBAA. Mustafa has presented a number of brewing-related papers at industry conventions at IGB, ASBC, and MBAA.

O-18

Precoat Filtration, Not a Dead End Street: Introduction of a New Generation of Candle Filters

THOMAS A. WEIGAND (1), Jürg Zuber (1), Ralf Brandau (2), and Cristian Rusch (2)

(1) Filtrix AG, St. Gallen, Switzerland; (2) Filtrix North America

Recent developments in the area of beer filtration, namely the introduction of a number of cross flow filtration systems on an industrial scale may suggest that conventional beer filtration with precoat filters using DE and/or other filter aids may become obsolete soon. This paper will highlight recent developments in the design of candle filters as commonly used throughout the brewing industry and review results from the industrial-scale introduction. The developments include the following: 1) Increased filter area per vessel. New production techniques allow the reduction of the diameter of the filter candle. This increases the effectiveness by achieving a higher packed density within a given filter vessel, thereby reducing dead volumes by approximately 20%. 2) Reduction of cleaning fluid consumption and beer losses. A new vessel shape and an adapted cake discharge process allow further reductions of cleaning fluid and beer water interface volumes. 3) Improved cleaning system. Cake discharge and internal cleaning can be improved by replacing the conventional spray bar. This feature turned out to be especially beneficial in PVPP filter applications. 4) Creating an open system. Further enhancements center around the flow pattern inside the filter vessel. By introducing a dual-path inlet distributor with adjustable flow rates, the upward and sideward flow direction inside a filter vessel

can be adapted to a wide variety of different filter materials such as different DE grades or alternative materials, such as cellulose, perlite, or the recently introduced synthetic polymers. This ensure that the candle filter will not become obsolete even in case DE may be phased out in the future. 5) Introduction of a new precoat material. A new preformulated precoat material, Celtrox PC, was introduced to reduce precoat quantities and reduce the number of precoats necessary to only one. At the same time, the cristobalite content is reduced to below 1%. In recent industrial applications, reduced haze readings during filtration and zero yeast counts throughout the whole filter run could be achieved. Quantities applied were 800 g/m² filter area, a reduction of 44% against the conventional process.

Thomas Weigand received a diploma in brewing technology from the Technical University Munich-Weihenstephan in 1983. He has been working as a sales and project engineer for beer filtration applications since 1985. In May 1999, he joined FILTROX AG, where he looks after the Americas as a key account manager. His hobbies are his three children, motorbikes, cooking, and skiing.

TECHNICAL SESSION IV: Hops

Moderator: Dave Hysert

As vice president, technical director for John I. Haas, Inc., in Yakima, WA, since 1992, David Hysert is responsible for research and development (R&D), technical services, and quality assurance (QA). Prior to joining Haas, he enjoyed an 18-year career at Molson Breweries of Canada, where he held various positions in R&D, technical services, and QA, including vice president, research, and QA from 1985 to 1992. He received a Ph.D. degree from the University of Toronto in bioorganic chemistry in 1971. David is an active member of many professional societies including the MBAA, ASBC, and Institute and Guild of Brewing. He was president of the ASBC in 1998–1999.

O-19

Organoleptic Profiling and Interactions of Hop Oil Fractions in Various Beer Types

RAY MARRIOTT
Botanix Ltd.

The chemistry of hop oil and hop oil fractions has been extensively studied over the last two decades but their organoleptic properties, their application in various beer types, and their interaction with other beer flavor molecules is less well understood. Trials have been carried out with analytically defined soluble hop oils and hop oil fractions in a range of beer types to determine the negative and positive changes in organoleptic profile using an expert taste panel. These can be expressed numerically and graphically to demonstrate the impact of hop aroma products on key beer flavor and aroma characters. The results have shown that it is possible to both enhance positive beer aroma notes, such as “citrusy” and “floral”, and also mask or reduce negative flavor characters, such as “worty”. Combinations of hop oil fractions can be used to create a new flavor profile to meet changing market requirements or to adjust current products to accommodate changes in manufacturing. The work that has been carried out also shows that some volatile hop aroma molecules interact synergistically with nonvolatile isomerized alpha-acids and modify the perceived bitterness and flavor/aroma balance. Further work is being undertaken to precisely identify the molecules responsible for this interaction. It is concluded that a better understanding of the organoleptic impact of hop oil fractions can assist brewers in the creation of new and enhanced products using an alternative and versatile source of hop aroma.

Ray Marriott received his B.Sc. degree (Hons) in biochemistry from Cambridge University and his Ph.D. degree in terpene chemistry from Bath University. After 20 years of experience in essential oil chemistry, he joined Botanix Ltd. (formally English Hop Products) in 1996 as its chief executive. He is a member of the IGB and has a particular interest in terpene enzymology.

O-20

Bitter Quality of Beer as Affected by Isocohumulone Levels

THOMAS H. SHELLHAMMER (1), Alix I. Gitelman (2), and Mina McDaniel (1)

(1) Department of Food Science & Technology, Oregon State University;
(2) Department of Statistics, Oregon State University

The quality of hop bitterness is a subtle but powerful driver of beer quality and contributes significantly to the “drinkability” of the final product. Anecdotal reports indicate that varieties high in cohumulone lead to an inferior bitter quality in beer. The objective of this study was to identify the impact of isocohumulone levels on the bitter quality of beer. A commercial beer with low inherent bitterness, Michelob Ultra, was spiked with pre-isomerized alpha-acid extracts that varied in isocohumulone levels. An extract from the variety Topaz was high in isocohumulone (~52% of total iso-alpha-acids), while Horizon was the source for low isocohumulone (~20%). The extracts were spiked in Michelob Ultra to yield an additional 10 and 20 ppm of total iso-alpha-acids and then compared by a panel of 14 experienced tasters using time intensity (via CompuSense data acquisition) and Free Choice Profiling descriptive technique. To eliminate olfactory influences, panelist wore nose clips throughout training and data collection. For the purpose of comparison, tetrahydroiso-alpha-acids and dihydroiso-alpha-acids were individually spiked at levels yielding roughly equal bitterness to the +20-ppm Topaz/ Horizon samples. Using panelists as blocks in a randomized block design, data were collected from four to five independent replications. In general, the differences in bitter quality between the high and low isocohumulone samples were subtle, with some panelists clearly differentiating the two, while many not. As a point of comparison, differences within the high and low isocohumulone samples were less than the difference between these samples and the tetra and rho extracts. More specifically, the relationships between concentration and each of peak intensity, duration, and time to maximum intensity were not different between high and low isocohumulone levels. Paired t-tests of time-intensity parameters indicated that the low isocohumulone extract had greater peak bitter intensity than did high isocohumulone. From the Free Choice Profiling study, the low isocohumulone extract appeared to be harsher than the high isocohumulone extract; this result was likely related to higher bitterness intensity and lingering qualities. The tetra sample was significantly different from all other hop extracts, with high bitterness plus harsh and lingering qualities. While the impact of isocohumulone levels on bitter quality appears very subtle, our results do not rule out the possibility that other, non iso-alpha-acid components in high cohumulone hops may contribute to harsh bitter quality.

Thomas Shellhammer is associate professor of brewing and food engineering in the Department of Food Science at Oregon State University. He received his B.S. degree in fermentation science and his Ph.D. degree in food engineering from the University of California, Davis. He currently serves as member of the ASBC Foundation Board.

O-21

Analysis of Hop Terpenes in Beer and Wort Using the SBSE Method with GC-MS

TORU KISHIMOTO, Noboru Kagami, and Katsuyuki Kawatsura
Asahi Breweries, Ltd.

Hop aroma components contribute to the aroma character of beer. For the analysis of hop aroma components, pretreatment is necessary. This involves steam distillation, extraction with conventional solvents such as dichloromethane, solid phase extraction, or purge and trap extraction. However, they each require time and labor and are somewhat inconvenient. We employed the stir bar sorptive extraction (SBSE) method with GC-MS as a very sensitive and easy method. This extraction method requires a very small quantity, 4–30 mL, of wort or finished beer. In addition, very few impurities are extracted. We determined main hop terpenes, linalool, geraniol, citronellol, myrcene, caryophyllene, humulene, humulene epoxide 1 and 2, alpha-eudesmol, beta-farnesene, and beta-damascenone in finished beer or wort. A low cross-validation value (below 10%) and a high correlation between the peak area and the internal standard ratio (over 0.99) were obtained for each substance. With this method, we traced the behavior of these terpenes during the wort boiling process. From our results, the decreasing pattern of terpenes are largely divided into two: one is substances that decrease gently and linearly, and the other is substances that decrease rapidly, drawing a quadratic curve. So, the hop terpene concentration determined by this method reflects the time hops are added and, in part, the hop variety.

Toru Kishimoto received his M.S. degree in agricultural chemistry from Kyoto University, where he majored in molecular biology of wheat protein. He began employment with Asahi Breweries, Ltd. in April 1999 in

the beer development section. Since September 2000, he has been engaged in hop research, especially in hop aromas.

O-22

Utilization of the Polyphenol Fraction from Hop Bract Part as Functional Food

MOTOYUKI TAGASHIRA, Msami Kurumatani, Rumi Fujuta, Yoko Akazome-Nagasako, Tomomasa Kanda, and Mitsuo Ikeda
Fundamental Research Laboratory, Asahi Breweries, Ltd.

As a worldwide tendency in recent years, although beer production is slightly increasing, demand of alpha-acid is not going up. The total hop crop continues to decrease because of low alpha dosage for beer and breeding improvement of bitter hop species. Indeed, statistics data suggested that the acreage of hop in 2003 was 55,029 ha, which was only 57% of that in 1992 (95,535 ha). In these situations, it will be meaningful to study the new way of hop utilization for nonbrewing applications. Hop bract part is a by-product discarded from the hop concentration process (making process of hop type 45). This by-product does not contain bitter acids, so it is thought to be a useless part for brewing beer. Therefore, most hop bract part is pelletized and used for cattle feed, but it may contain something useful. In this paper, we report that the valuable utilization of hop bract part as a functional material of food. We developed the method to extract and separate the polyphenol fraction from hop bract part. The polyphenol fraction can be used for food materials because this method uses only water, ethanol, and materials permitted for industrial food processing. This polyphenol fraction is characterized by its rich content of high-molecular-weight polyphenols, which are presumed as highly condensed catechins. Although hop has been used as food material and thought to be safe, we also checked the safety of this fraction by several assay methods, such as acute (14-day and 28-day) toxicity and mutagenicity. Recently, some studies have shown that the polyphenol fraction had several activities such as antidental caries and antibacterial toxins. For example, this fraction showed potent anticavity activity through inhibiting the biofilm-making enzyme produced by *Streptococcus* bacteria (M. Tagashira et al., *Biosci. Biotech. Biochem.* 61:332-335, 1997). These studies are responsible for the potential of this polyphenol fraction as not only food addition for taste improvement, but also as a functional food material. It will be possible to develop the anticavity gums (or candies) that contain this polyphenol fraction. This finding shows the possibility of industrial utilization of hop bract part. Through this study, we hope to contribute to the development of a new aspect of hop utilization that is not limited to only brewing purposes.

Motoyuki Tagashira received a master's degree from Tokyo University in Tokyo, Japan. He began employment with Asahi Breweries, Ltd. in 1992 as a research scientist. He is working primarily on developing new food materials that have positive functions for human health.

O-23

A Proteome Approach for Detection and Characterization of Hop Inducible Proteins Involved in Hop Resistance of Beer-Spoiling Lactobacilli

JÜRGEN BEHR, Michael G. Gänzle, and Rudi F. Vogel
Technische Universität München, Freising, Germany

The resistance to hop is a prerequisite for the capability of lactic acid bacteria to spoil beer. Therefore, the knowledge on the hop resistance mechanisms allows the specific and sensitive detection of beer spoiling lactic acid bacteria, and may enable the evaluation of targets for mild preservation techniques. Several enzymes involved in hop resistance of lactobacilli have recently been characterized on biochemical and genetic levels. However, the enzymes characterized do not fully account for the hop resistance of lactic acid bacteria. Additional hop resistance mechanisms are known to be involved in beer spoilage. To obtain a global view on the response of lactobacilli to a challenge with hop, a proteome approach was taken to determine hop inducible proteins that contribute to the hop resistance of *Lactobacillus brevis*. The highly hop-resistant beer-spoiling isolate *Lactobacillus brevis* TMW 1.465 was cultured in modified MRS to the exponential growth phase at pH 6 (reference conditions), pH 4.0 (acid stress), and pH 4.0 in the presence of hop (86 µmol/L isohumulone; hop stress). For cells from each condition, extraction procedures were performed for cytoplasmic proteins, as well as enhanced recovery of membrane proteins. For proteome analysis, two-dimensional polyacrylamide gel electrophoresis with immobilized pH gradients was

applied. The identified hop inducible proteins were blotted on a PVDF membrane and are being sequenced. A reference map with proteins of *L. brevis* expressed at pH 6 was established and compared with those proteins expressed by the same strain under conditions of acid stress or hop stress. More than 20 proteins were overexpressed more than 1.5-fold in the presence of hop as compared with the reference. The majority of these hop inducible proteins were present in the membrane protein-enriched fraction only and were selectively induced by hop. The induction of hop inducible cytoplasmic proteins was below detection level. Sequencing of hop-induced proteins is currently underway to obtain a global view on the cellular response of beer-spoiling lactobacilli to challenge with hop compounds. The genes coding for hop inducible proteins are identified by reverse genetics, and novel hop resistance mechanisms identified by this proteome approach are characterized on a biochemical level.

Jürgen Behr was born in 1974 in Kulmbach, Germany. During his study of "Technology and biotechnology of foods" in Weihenstephan, Freising, Germany, he acquired experience in food science as well as brewing technology. After projects in the field of yeast proteomics and the physiological basics of lactobacilli (beer spoilers), he concluded his study with a master's thesis about high-pressure resistance of beer-spoiling lactobacilli to obtain the degree of a Dipl.-Ing. Currently, he is working on his Ph.D. thesis at the Chair of "Technische Mikrobiologie" (Technische Universität München). The investigations are focused on the molecular mechanisms of hop resistance of beer-spoiling lactobacilli.

O-24

Potential of Reutericyclin as a Tasteless Hop Analogue in Beer Preservation

Clarissa Schwab, Michael G. Gänzle, and RUDI F. VOGEL
Technische Universität München, Freising, Germany

Reutericyclin is a heat-stable, low-molecular-weight, antibacterial compound produced by cereal isolates of *Lactobacillus reuteri* that shares structural similarity to hop iso-alpha-acids (1). It is active toward gram-positive bacteria but inactive toward yeasts. Various species of thermophilic lactobacilli are employed in sour wort fermentations to improve beer flavor and to adjust the wort pH to the desired level. Selected strains with antimicrobial activity may be used in sour wort fermentations to additionally contribute to beer preservation. In this work, the mode of action of reutericyclin toward beer-spoiling lactic acid bacteria was characterized. Its formation was determined by *L. reuteri* in wort. The mode of action of reutericyclin toward a beer-spoiling *Lactobacillus plantarum* was compared with the known mode of action of hop iso-alpha-acids. Based on its structural similarity to hop iso-alpha-acids, the cytoplasmic membrane was examined as a major target for reutericyclin activity. The fluorescent dyes propidium iodide, carboxyfluorescein-diacetate, and dipropyl-thiadiazocarbocyanine-iodide were employed to determine the effect of reutericyclin on the integrity and the proton and potassium gradients across the membrane, respectively. Comparable to hop iso-alpha-acids, reutericyclin acted as proton-ionophore, thereby dissipating the proton gradient across the membrane of sensitive cells in a pH-dependent manner. The inhibitory effect of reutericyclin alone or in combination with hop extracts was determined toward two beer-spoiling isolates of *L. plantarum* and *Lactobacillus brevis* exhibiting intermediate and high hop resistance, respectively. Using either indicator strain, reutericyclin could functionally substitute hop compounds. Furthermore, hop resistance of these two strains correlated to the reutericyclin resistance, indicating that hop resistance in beer-spoiling lactobacilli confers reutericyclin resistance. Fermentations with *L. reuteri* in wort were carried out at static-pH conditions to determine whether reutericyclin is formed in cereal substrates. It was demonstrated that reutericyclin is produced in active concentrations during growth of *L. reuteri*. Optimal pH values for reutericyclin formation ranged from 4.0 to 5.0. In conclusion, reutericyclin is produced to active concentrations during growth of *L. reuteri* in sour wort fermentation and is active toward beer-spoiling lactic acid bacteria. Therefore, reutericyclin-producing strains of *L. reuteri* have a potential for use as biopreservatives in brewing applications. (1) Gänzle et al. 2000. *Appl. Environ. Microbiol.* 66:4325.

Prof. Dr. Rudi F. Vogel was born in 1955 and is a biochemist (Universität Tübingen, Germany) interested in food microbiology and biotechnology. Since his habilitation on the genetics of lactobacilli (Universität Hohenheim, Germany), he is head of the Technische Mikrobiologie in the

Department for Food and Nutrition of the Technische Universität München, Germany. He supervises and coordinates research on lactic starter culture development ranging from ecology and biochemistry to functional genomics, including several projects in brewing science and high pressure in food and biosciences. He is a member of the editorial board of scientific journals, international associations, and advisory committees on food safety and genetically engineered organisms.

O-25

A Rapid and Low-Cost Method for Quantification of Reduced Iso-Alpha-Acids in Brewing

Alexis Bolívar, MÓNICA GASPARRI, and Carsten Zufall
Cervecería Polar, C.A.

Reduced hop extracts (rho-, tetrahydro-, and hexahydro-iso-alpha-acids) are in widespread use in breweries around the world for the purpose of achieving light-stable products with foam and cling enhancement. Brewers can take advantage of blending these iso-alpha-acids in order to produce beers with different flavor profiles. The traditional UV bitterness units (BU) method cannot be used for quantifying the components within a composition of reduced iso-alpha-acids. The HPLC method is currently the only analytical tool for controlling hop-dosing and bitterness in beers brewed with reduced iso-alpha-acids. Furthermore, it permits detection of iso-alpha-acid contaminations in light-stable beers. However, it is much more expensive and time-consuming than the BU method. Production of light-stable beers typically requires hop dosing during filtration. Thus, in large-scale production, time is very limited for BU measurements before and after dosing. Initially, we used the HPLC method with sample pretreatment by manual solid-phase extraction (SPE) and analysis by C-18 reverse-phase column with a relative standard deviation (RSD) 1.6%. We have developed a novel HPLC method for routine analysis, making it less expensive and faster by direct injection using a new-technology HPLC column, Chromolith™. The 60 min required previously has now been reduced to just 8 min. This has resulted in an 86% savings in time and a 60% savings in cost. The method permits direct analysis of wort or beer after disc membrane filtration. It leads to the calculation of a profile concentration of the different iso-alpha-acids. Finally, with the varying relative bitterness intensity, it yields organoleptic bitterness units. The improved method with RSD 0.9% has been validated and implemented in all five of our laboratories. In order to fulfill the validation requirements, we have tested linearity, accuracy, precision, specificity/selectivity, range, and ruggedness/reproducibility, as well as detection limit.

Mónica Gasparri is chromatography coordinator in the Corporate Laboratory at Cervecería Polar C.A. During her 20 years at Polar, she has managed various responsibilities in the chromatographic area. Monica received her Docteur de Troisième Cycle in organic chemistry in the Sciences Faculty of Poitiers University, France.

TECHNICAL SESSION V: Environmental/Engineering

Moderator: Kathy Kinton

Kathy M. Kinton began her career in the brewing industry at Miller Brewing Company in 1979. She has worked in various positions in quality services and corporate environmental engineering, and she became the quality services manager at the Irwindale Brewery in 2001. Kathy joined MBAA District Milwaukee in 1988 and has served as district president in 1994 and 1995. She served as chairperson of the MBAA Scholarship Committee from 1993 to 1995 and chair of the MBAA Education Committee from 1996 to 1998. Kathy has been an instructor for MBAA courses and authored the chapter, "Environmental Issues Affecting Brewery Operations" in the new edition of *The Practical Brewer*. She has presented various papers on environmental issues and facilitated the first Environmental Workshop at the 2001 ASBC Convention. Kathy was MBAA president in 2001 and is cochair of the WBC 2004. She also is a member of the ASBC Foundation Board. Kathy received her bachelor of science degree in food science from North Carolina State University in Raleigh, NC, in 1973 and is a graduate of the 1979 MBAA Brewing and Malting Science Course.

O-26

ECO-MATRIX: A New Economical Pipe System

KRISTINA BOEE

Tuchenhagen Brewery Systems, Buechen, Germany

Vision becomes reality. ECO-MATRIX... The new, efficient piping concept for process plants! The new Tuchenhagen piping system, ECO-MATRIX, offers cost effectiveness and efficiency so far unreached in systems engineering. By comparison to ordinary systems, ECO-MATRIX reduces considerably the number of instruments required and allows for essential optimization of process sequences. This helps you to manage your capital investment and provides a much faster pay-back. Production processes in modern brewery and beverage plants are determined increasingly by economic factors and product quality requirements. There is a continual requirement to improve product quality, increase operational safety, and at the same time, reduce capital and operating costs and minimize product losses. Quite a challenge! The response to this challenge is ECO-MATRIX. ECO-MATRIX is a new system of piping from Tuchenhagen in which the process pipes and process valves are connected directly beneath the tank outlet. The process valves may be arranged either laterally at the tank outlet tree, at the tank cone, or vertically at the tank bottom flange. This innovative system significantly reduces the length of piping required, thereby reducing product losses and minimizing the risk of contamination during the brewing or distribution processes. The technical advantages of ECO-MATRIX at a glance. • Simple and quick to install. • Reduced space requirement. • Short tank outlet pipes, minimized product losses. • No dead ends at the tank outlet.

Kristina Boee is head of engineering international. She holds a B.Sc. degree in process engineering and a Dipl.-Ing. from the Technical University Hamburg-Harburg, Germany. Kristina Boee has 6 years of experience in the beverage and brewing industry. Her personal project contributions include project engineer for process units (wort aeration and yeast pitching); product manager for process units (carbonation, mix-processing, deaeration, wort aeration, and yeast pitching)—presentations, sales material, support, technical developments; project manager for beverage plants (complete integration, new developments, design, engineering, installation, and start up); technical support for Tuchenhagen North America and process units (55 units, 1995–1999, each 0.1–0.5 Mio. Euro; Lasko Brewery/Slovenia, two plants, 1997–1999, 0.5–1 Mio. Euro; and Union Ljubljana Brewery/Slovenia, one plant 1998–1999, 3 Mio. Euro). Kristina studied until 1995. She then worked for Tuchenhagen Brewery Systems GmbH, Germany, as product manager (1996–1998), project manager (1998–1999), and head of engineering international (2000–present).

O-27

Asahi's Approach to Reduction of Energy Basic Unit to Half

AKITOSHI YOSHIZAWA

Asahi Breweries, Ltd.

In December 1997, the COP3 conference for the prevention of global warming was held in Kyoto, and a worldwide agreement was reached regarding an approach to addressing the problem. In Japan, there is also a growing recognition that society as whole must make a concerted effort to further curb CO₂ emissions. In response to such a trend, Asahi Breweries decided to tackle the task to reduce the energy basic unit to half. The amount of CO₂ emission resulting from fuel and electricity consumption was 70% of all the CO₂ emitted at the breweries. Therefore, the reduction of fuel and electricity consumption would be greatly effective for energy saving and also for CO₂ reduction. This paper will introduce the approaches to energy saving at Asahi Ibaragi Brewery. Until 2000, 4 years ago, the energy basic unit of Ibaragi Brewery was rather high, 134 MJ/hL in fuel and 166.5 MJ/hL in electricity, (= 16.26 kWh/hL × 10.24 MJ/kWh as in the calculation method of Japanese Law concerning the Rational Use of Energy). Due to our energy-saving efforts, in the 2003 year-end, the fuel basic unit became 103 MJ/hL, down 23% from the year 2000, and the electricity basic unit became 117.4 MJ/hL (11.46 kWh/hL), down 30%. Furthermore, in 2006, we will try to achieve an energy basic unit of 121 MJ/hL in fuel and 37.7 MJ/hL (3.68 kWh/hL) in electricity, and to introduce the 5,000-kW cogeneration system. Regarding the approaches, first we created the energy balance sheet on fuel and electricity to grasp the situation. In clarifying the problems, we compared Ibaragi Brewery with Nishinomiya Brewery, which was marked as our top energy-saving brewery. This gave Ibaragi Brewery the top level of energy basic unit among our breweries. Second, we calculated the theoretical energy basic unit and compared it with Ibaragi's improved energy balance sheet to pinpoint further problems. These two steps made us promote energy-

saving approaches effectively. One example of actual measures is an establishment of a heat exchanger that utilizes the cool thermal energy before the CO₂ vaporizer. The previous CO₂ vaporizer used steam to heat up and vaporize CO₂ liquid of -7.6°F to 86°F. Ibaragi Brewery used to use 14,000 t of CO₂ and more than 6 million MJ of fuel per year. By establishing the heat exchanger with propylene glycol before the CO₂ vaporizer, we reduced electricity consumption of a freezer and the amount of required heat for the vaporizer. This resulted in a reduction of 5.15 million MJ of fuel and 0.45 million kWh of electricity per year. Other majors taken between 2001 and 2003 rose to seventy, with 31 MJ/hL in fuel and 49.2 MJ/hL (4.8 kWh/hL) in electricity reduced as a total.

Akitoshi Yoshizawa received a B.S. degree in mechanical engineering from Meiji University in Tokyo, Japan. He began employment with Asahi Breweries, Ltd. in April 1995 as an engineer in the brewery. He has worked for about 7 years as an engineer (brewing, packaging, etc.). Since September 2001, he has approached the reduction of the energy basic unit to half in the R&D Promotion Office of Asahi Breweries, Ltd.

O-28

Membrane Separation Activated Sludge Processes: Method of Purifying Warm Water for Warmer

YASUHIRO SASAKI

Asahi Breweries, Ltd.

Asahi uses the device, Warmer, that warms cans or bottles to normal temperature by circulating warm water after filling with beer, because dew condensation on beer containers affects the rest of the processes. This circulated warm water gets dirty by beer components clinging onto beer containers during filling. Therefore, we used to do a daily job of exchanging water and washing Warmer. The new method, membrane separation activated sludge processes, made it possible for us to keep it clean with only a once-a-week job. This enabled us to improve production efficiency with longer operation hours and to cut down utility expenses. Furthermore, processed water with this method has a high quality and a possibility of reuse in another processes, which we investigated for a closed system. Membrane separation activated sludge processes is the method that simultaneously performs both biological processing and filtration with microfiltration membranes whose 0.4- μ m surface is where microbes are adhered. It has two features: a simple composition and easy maintenance. This device is composed of an activated sludge tank, membranes filtration units, a pump, and a blower for aeration. We set it up at a working can line and used this processing method to purify circulated warm water in Warmer. Regarding the processing conditions, the quantity of Warmer's holding water is about 9 m³, the pollution rate of circulated warm water is between 5 and 12 mg/L/h as COD, the processing capacity is between 2 and 4 m³/h, the device volume is about 5 m³, the purifying method is consecutive processing, and the temperature is from normal temperature to 45°C. As for the result, the quality of wastewater is changed through the method. Between 30 and 180 mg/L as COD in wastewater is reduced to be 10 mg/L or less. The turbidity, ranging between 2.0 and 9.5 NTU, in wastewater becomes 0.15 NTU or less. pH ranging between 5.3 and 7.0 becomes between 5.8 and 7.8. Unusual odor and color of wastewater becomes usual. The number of bacteria in the standard plate count is greatly reduced, from more than 8×10^4 CFU/mL to less than 200 CFU/mL to nil. Regarding the water quality in Warmer after the five-consecutive-day performance of the method, COD becomes 60 mg/L or less, the turbidity becomes 4 NTU or less, pH ranges between 5.6 and 7.3, and both odor and color become usual. These results show that the water processed with this method becomes pure and that the method is useful. Therefore, we think it is possible to continue to use the processed water for a much longer time, without disposing it.

Yasuhiro Sasaki received a B.S. degree in chemical engineering from Chuo University in Tokyo, Japan. He was employed with Asahi Breweries, Ltd. in August 1997 as an engineer in the brewery. He has worked for about 6 years as an engineer (brewing, packaging, etc.). Since September 2002, he has been researching membrane separation activated sludge processes in the Research and Development Promotion Office of Asahi Breweries, Ltd.

O-29

Happy Fish Due to or In Spite of an Optimized Wastewater Treatment System?

VERA GROOT KORMELINCK (1), Bernd Franzmann (2), and Shashi Gorur (3)

(1) Paques, The Netherlands; (2) Karlsberg Brauerei, Homburg, Germany; (3) USFilter, U.S.A.

The importance of an excellent effluent quality has to be met by the Karlsberg Brewery in Homburg, Germany. They discharge their purified wastewater into a small river. A necessary condition for discharging into this river is that there should be no ecological damage to the existing ecosystem. Until the late 1990s, the Karlsberg Brewery discharged their wastewater to the municipal wastewater treatment plant. Economical factors and the belief that the stringent effluent demands could be met with an on-site wastewater treatment plant made Karlsberg decide to build their own. This paper presents the historical background of the project and a description of the technologies applied. Operational data, including any problems that occurred during startup are discussed, as well as the economical benefits of the wastewater treatment plant. This enables an overview of the current state of art in the treatment of brewery effluent.

Vera Groot Kormelinck graduated in food technology at the Friesland College of Food Technology in 1989. After graduation, she started her professional career at the laboratories of Vriezo bv. Her employment with Paques bv began in 1989, where she lead the biological startup of a large demonstration project at Heineken in Den Bosch. In 1996, having served several years as a startup engineer at various industrial effluent treatment projects, she joined the process engineering department of Paques. In 1998, she started her function as a proposals manager, in preparation for a more commercially oriented career. In 2000, she became area sales manager for Germany with a focus on the beer and beverage industry. At the beginning of 2004, she accepted responsibility as branch manager, beer and beverage market, for Paques.

O-30

Best Available Techniques in the Brewing Industry

P. W. VAN OEVEREN

Heineken International B.V.

Introduction: The European Union Council Directive 96/61/EC concerning integrated pollution prevention and control (IPPC) came into force in October 1996. The purpose of the directive is to achieve a high level of protection of the environment through an integrated approach to prevent emissions to air, water, or soil. The IPPC permit shall include measures based on the so-called Best Available Techniques (BATs). All breweries with an output of over 1 million hL per year shall have an IPPC permit in 2007 ultimately. The role of the Best Available Techniques in the brewing industry will be described in this presentation. A Best Available Technique (BAT) is defined as being the most effective ("Best") and accessible technology on a scale that allows implementation under economically and technically viable conditions ("Available"). A BAT is not a single solution but depends a.o. on local environmental conditions. The directive promotes the dissemination of such techniques by maintaining so-called BAT Reference Documents or BREF. One of those BREFs covers food, milk, and drink, including breweries. Branch organizations, such as the CIAA and the Brewers of Europe, had an important contribution to this BREF. Challenges ahead: The Brewers of Europe have followed the BREF development very closely and anticipated it by preparing a Guidance Note for establishing a BAT in the brewing industry. The reason was to summarize the 520-page BREF into a document of 30 pages, not only to help the breweries understand the BREF but also to explain to the permitting authorities what the environmental impact of breweries comprises. Another important factor is the assessment of the environmental benefits against the investments. The Technical Working Group "Economics and cross media effects" is investigating the ingredients to improve objectivity, transparency, and consistency of the decision-making process. The Brewers of Europe have developed a decision tree to evaluate candidate BATs. The stepwise approach assesses the technological feasibility of such things as beer recipe and product safety. These subjects should be valued against the environmental benefits. The economic consequences should be based on feasibility and affordability. Conclusion: The IPPC Directive refers to the BREFs, which provide an instrument to consider measures concerning a broad range of environmental impacts. The breweries should be aware of the degrees of freedom and to be alert to negotiate with the authorities the proper permit. The Guidance Note of the Brewers of Europe should help

the breweries in this process. The EBC Technology & Engineering Forum will start a Working Group BAT to keep the Guidance Note up-to-date.

Pjotr van Oeveren received an M.Sc. degree in chemical engineering from the Technical University in Delft, The Netherlands. He currently works with Heineken International B.V. and has worldwide responsibility for safety and environment policies. He is a member of the IPPC-BAT committees of CBK, Brewers of Europe, CIAA, EBC, and the Technical Working Group.

O-31

Greener Beverage Product Security and De-Casing Solutions

PAUL LIGON and Neal Gutkin
WM IPS

This presentation will present a strategic alternative to beverage destruction sourcing that emphasizes cost-effective resource efficiency through on- and off-site product destruction, liquid recovery, and container recycling practices. The presentation will assess the product security and de-casing industry in the context of several established and emerging business trends, including performance-based contracting, outsourcing of “non-core” functions, strategic partnering, and the movement from leveraging physical capital to intellectual (knowledge, information, and learning) assets. Each of the points below will be elaborated on in more detail. 1) The de-casing industry structure and fit within the brewing industry value chain, and how de-casing contracts can be structured to produce superior services and mutual, profitable gains. 2) Models and case studies of effective de-casing and product security operations and relationships that produce tangible savings, security value, and environmental results. 3) Overcoming the purchasing mentality. - Appropriating value from the product destruction and de-casing supply chain. - How to ensure product security and de-casing services are sourced as a systems buying practice. The presentation will provide a thorough analysis of these points while also providing practical information and resources for those wishing to take this emerging model to the next level.

Paul Ligon is a business development manager with Waste Management's In-Plant Services Division (IPS). IPS is a leading provider of environmental sourcing solutions to a wide range of industries. Between 1990 and 2001, Mr. Ligon was a senior scientist at the Tellus Institute, a global environmental research and consulting firm in Boston. In this capacity, he has advised companies and governmental agencies in the U.S., Australia, Central America, and Egypt on environmental strategies related to supply chain initiatives, information systems, reporting and disclosure, financing, accounting, and project management. He is widely published in environmental trade and academic journals and speaks regularly at professional conferences on environmental best management practices. Paul holds an M.B.A. degree from the Tuck School of Business at Dartmouth and a B.S. degree from the University of Vermont.

O-32

Aware of Water

PAUL J. M. BRUIJN, Pjotr W. van Oeveren, and Sietse W. Montijn
Heineken

Heineken is a worldwide brewer with more than 130 breweries in 60 countries. During the last 20 years, Heineken has monitored the water supplies for these breweries. We have seen lower water tables, have closed wells due to chemical contamination, and in one extreme case, closed a brewery for 2 months during a severe drought due to no water. In 1999, due to these concerns and in support of our Environmental Policy, we developed a global Water Policy. This policy resulted in a four-part Aware of Water program. 1) Documentary film series “Water the Drop of Life”. Heineken supported the production of 13-part documentary series on water. Each part is 50-min long and is intended to increase awareness of water use by the general public. This television series has been broadcast in more than 100 countries. 2) CEO Panel of World Water Forum. Heineken's CEO is a member of the World Water Forum CEO Panel. The panel represents a range of businesses, with a shared commitment to water management. In 2001, the Panel issued a statement addressing four major water themes and, in 2003, reviewed the results of five significant water projects. 3) Water usage. Water use by beer production chain was quantified with a life cycle assessment. This assessment began with barley growing, ended with water use by consumers, and included water use for packaging, malting, and brewing. Barley growing used the most water,

about 200 hL of water for every hL of beer (200 hL/hL), which was largely rainwater. Clearly, brewers have difficulty in reducing this use. Breweries used, on average, about 10 hL/hL. This use can be influenced by brewers. Next, a benchmarking study with both Heineken and non-Heineken breweries was carried out. The study showed that water use varied greatly, from a low of 4 hL/hL to a high of 24 hL/hL and also related water use to volume, location, and packaging mix. Based on this study, Heineken established a maximum water target of 7 hL/hL for every brewery, irrespective of size, location, or packaging mix. 4) Reduced water usage in Heineken Breweries. To meet this target, an educational workshop, “Aware of Water”, was developed. The outcome was a water savings plan for each brewery, developed by the people from that brewery. These plans were implemented and the results recorded by Heineken environmental reporting system. In the 3 years since these workshops were held 1) all Heineken breweries have reduced water use, 2) the weighted average water use has been reduced by 1 hL/hL, and 3) 50% of the breweries that were above the target now meet the target. Based on the success of the Aware of Water program, a second edition is being developed for breweries that have not yet met the target and an Aware of Energy program is being carried out.

Paul Bruijn received a degree in biology from the University of Leiden, The Netherlands. He began his employment with Heineken in 1989 as a research scientist at Heineken Technical Services. Research topics included anaerobic and aerobic wastewater treatment, cleaning and disinfection, and valorization of brewery coproducts such as brewers spent grains and surplus yeast. Since 1996, he has functioned as an environmental specialist and has been involved in various improvement programs including “Aware of Water”. Furthermore, he is involved with internal and external safety & environmental reporting for the Heineken maltings, breweries, and soft-drink plants.

TECHNICAL SESSION VI: Malting

Moderator: Xiang Yin

Xiang S. Yin is the technical manager for Cargill Malt Americas, based at Prairie Malt Limited, Canada. He obtained his first degree in engineering in fermentation technology at Wuxi, China, and received his Ph.D. degree in 1986 from Heriot-Watt University, Edinburgh. He carried out his postdoctoral research at the University of Edinburgh and then at the Grain Research Laboratory in Winnipeg. As the recipient of the 1990 Centenary Research Award of the Institute of Brewing, Xiang worked at the Brewing Research International, England, on beer flavor in the same year. He was an associate professor at the Wuxi Institute of Light Industry in China for 3 years before joining Prairie Malt as director of technical services in 1991. Xiang is the author or coauthor of more than 30 scientific and technical papers. He recently served as the district executive and representative on the Board of Governors for District Western Canada of MBAA.

O-33

Raw Barley as Adjunct—Optimal Application of Malt and Commercial Enzymes for Beer Production

DECLAN L. GOODE (1,2) and Elke K. Arendt (1)

(1) Department of Food and Nutritional Sciences, National University of Ireland, University College Cork, Ireland; (2) National Food Biotechnology Centre, National University of Ireland, University College Cork, Ireland

In traditional brewing, malted barley is the grain of choice. It acts not only as a raw material, supplying starch and protein, but it also contains a sufficient supply of enzymes necessary for the efficient production of wort. However, in different parts of the world, barley-growing conditions may be poor, malting facilities and malting conditions are quite often less than optimal, and the importation of malted barley can be expensive. Considerable savings can thus be made by replacing part or all of the malted barley by unmalted cereals, such as raw barley, together with exogenous enzymes. Brewers are often in a dilemma as to the level of malt replacement and commercial enzyme addition that is possible without negatively affecting the quality of their products. The objective of this study was, therefore, to evaluate the effects of both endogenous malted barley enzymes and exogenous commercially produced enzymes on final wort quality when mashing with raw barley. Laboratory-scale trials were carried out to determine the effects of malted barley addition, when mashing with raw barley. Additional laboratory-scale brewing trials were

carried out in which a range of different commercial enzymes (proteases, alpha-amylases, and beta-glucanases) was added during the mashing process at different dosage rates. These enzymes, which were added in both cocktail form and individually, were assessed and characterized with respect to their effects on mash filterability, wort quality, and fermentation characteristics when mashing with raw barley as a substrate. With the addition of malt, increases in extract, fermentable sugars, free amino nitrogen levels, and fermentability were observed. Increasing the amount of bacterial protease also gave corresponding increases in free amino nitrogen. Commercial alpha-amylase addition yielded increases in the rate of filtration. However, at increased levels, negative effects on filtration were observed. Without the aid of malt enzymes, the inclusion of a commercial heat-stable alpha-amylase was necessary to yield a starch-negative wort. Commercial beta-glucanase addition was necessary at low levels to reduce wort viscosity and beta-glucan content of the wort. By comparing the data of the malt addition trials together with the data of the commercial enzyme addition trials, suggestions were made concerning barley brewing with the overall aim of maintaining or increasing wort quality while reducing costs.

Declan L. Goode received a B.Sc. degree in food technology from The National University of Ireland, Cork, Ireland, in 1998. He received his M.Sc. degree in the area of brewing at the National University of Ireland, Cork, in 2001. The title of his thesis was "Brewing with unmalted sorghum and commercial enzymes". He is currently employed as a senior research scientist at the Research Malting and Brewing Facility of the National University of Ireland, Cork, Ireland, where he takes responsibility for the running of the research brewery. He is also working toward his doctorate degree. His areas of research include enzymes and unmalted cereals. He has previously presented at international conferences and has recently published in the Journal of the Institute of Brewing and the Journal of the ASBC.

O-34

A New Technique for Combined Milling and Mashing in the Brewhouse

Gary J. Freeman (1), Michael Ruth (1), Michael Todman (2), and F. RICHARD SHARPE (1)
(1) Brewing Research International; (2) Pursuit Dynamics PLC

Currently, a brewery mash is performed using a sequence of operations. The malt is carefully milled to a suitable particle size distribution and mixed with other solid grist material as required. Brewing liquor is heated to a target temperature so that, when mixed with the cool grist, the correct starting temperature is obtained in the mash. The liquor and grist are then mixed using a masher that produces a homogenous suspension of the grist in the liquor. The work described assessed the feasibility of employing an innovative new technology to simplify this process sequence. The new process relies on a "PDX" unit, a patented system. The PDX is able to function simultaneously as a pump, mixer, heater, and macerator. Steam is injected into a stream of malt and liquor. The injection point comprises an annular ring around the pipe. With sufficient steam flow, the entry velocity is supersonic and the resultant shock wave is sufficiently energetic to macerate ("mill") the malt grains and disperse them. Some of the mash heating is provided by the steam injection. Potentially, a combined milling and mashing process based on PDX could enable savings in capital expenditure, energy, manpower, maintenance requirements, and space. The feasibility study presented was designed to investigate the potential applicability of PDX in the brewhouse. Initial work enabled a preliminary unit design to be selected. Subsequently, the technology was evaluated in a pilot plant (100-L brewlength). Results have been interpreted in terms of economic value, wort quality, and the selection of the appropriate wort separation technology.

Richard Sharpe obtained his first degree in chemistry. He then studied for his Ph.D. degree at the Brewing Research Foundation, where he investigated the chemistry of beer flavor, hop oil, and the extraction of hops and liquid carbon dioxide. He joined Whitbread plc in 1979 and, after a 20-year career in science and technology, left his position as director of beverage research and development to join Brewing Research International as their technical director, where he is responsible for the sales and marketing function. He is a visiting professor at Luton University and is the author of 60 publications and two patents. He is a fellow of the Royal Society of Chemistry, a fellow of the Institute of Food Science and Technology, and a fellow of the Institute of Brewing. He is

chair of the Institute & Guild of Brewing's Analysis Committee and a member of the Heriot Watt Research Committee.

O-35

Physicochemical Changes in Barley/Malt During the Malting Process with Particular Emphasis on Beta-Glucan and Beta-Glucanases

John O'Flaherty and EOIN LALOR
Quest International Ireland Ltd.

A detailed study on the effect of various germination programs on malt modification was carried out on European barley. Particular attention was paid to the effect of germination time and temperature on final malt quality as determined by EBC mashing experiments on a laboratory scale. Parameters measured were mash filtration efficiency, extract yield, and malt beta-glucanase and malt/wort beta-glucan content. Changes to malt beta-glucanase and malt beta-glucan (content and molecular weight) were monitored at different stages during the malting program. The information obtained gives a clear insight into malt modification and explains where and why problems might arise in subsequent processing in the brewery.

Eoin Lalor graduated from Trinity College Dublin with an honors degree in biochemistry. Having worked in medical research (cancer and multiple sclerosis) for a number of years, Eoin joined the Whitbread Brewing Company as a brewing research scientist. In 1991, Eoin joined Quest International as a senior scientist for their brewing products division. After a number of years working in company headquarters in Holland as an applications manager, Eoin returned to Ireland. Eoin is currently business development manager for brewing products working with all major brewing companies worldwide.

O-36

Malting Characteristics of Three Canadian Hulless Barley Varieties, CDC Freedom, CDC McGwire, and CDC Gainer

YUESHU LI (1), Rob McCaig (1), Aleksandar Egi (1), Michael Edney (2), Marta Izydorczyk (2), and Brian Rosznagel (3)
(1) Canadian Malting Barley Technical Centre, Winnipeg, MB, Canada;
(2) Canadian Grain Commission, Grain Research Laboratory, Winnipeg, MB, Canada;
(3) Crop Development Centre, University of Saskatchewan, Saskatoon, SK, Canada

Several malting trials at micro and pilot scales were carried out with Canadian hulless barley, CDC Freedom, CDC McGwire, and CDC Gainer, of the 2001 crop. Their malting behavior and malt quality were evaluated in comparison to a covered two-row malting variety, AC Metcalfe. Trial results indicated that all three hulless varieties could be malted successfully and produced quality malt under processing conditions that were tailored to each variety's requirement. The required total malt processing time was up to 7 days. In the trials, all three barleys showed rapid water uptake and rapid chitting, and the progress of modification was similar to that of the control AC Metcalfe. However, excessive acrospire damage by the turner during germination was recorded, which was attributed to the lack of husk protection. This suggested that turning frequency needed to be adjusted during germination to reduce the damage. These hulless barleys were more sensitive to kilning conditions, and varying the kilning regimes significantly affected malt quality, especially malt friability, more than it did in the control. In the trials, CDC Freedom, CDC McGwire, and CDC Gainer produced malt with very high extracts, about 2–4% higher than that of the control AC Metcalfe. Malt soluble protein and diastatic power were comparable to those of the control malting variety, while wort viscosity was higher and alpha-amylase level was lower. In comparison, CDC McGwire's pilot malt had more balanced quality, with the highest friability and lowest beta-glucan content among the three hulless varieties. In micromalting trials, the beta-glucan levels of the trials was reduced from that seen in the pilot-scale maltings, and the varietal differences seen in pilot malting trials disappeared. This suggested that the difference in malt beta-glucan content was mainly contributed by the difference in malting conditions. Wort analysis also indicated that the wort quality was comparable to that of covered barley in terms of sugar compositions.

Yueshu Li is director of malting technology at the Canadian Malting Barley Technical Centre in Winnipeg, Canada. He joined the Centre in August 2000. Previously, he was senior technical consultant for malting barley in the Market Development Department of the Canadian Wheat Board. Dr. Li has more than 14 years of malting industry experience and

has held several senior research and management positions in the malting industry in both North America and China, including *Prairie Malt Limited, Canada*; *Schreier Malting, U.S.A.*; and *CUC Nanjing Malt Limited, P.R.C.* Yueshu was born in China and educated in both China and Canada. He obtained his B.Sc. and M.Sc. degrees in China and a Ph.D. degree from the University of Saskatchewan, Canada. He is a member of MBAA, ASBC, and AACC.

O-37

Brewing with Canadian Hulless Barley Varieties, CDC Freedom, CDC McGwire, and CDC Gainer

ROBERT MCCAIG (1), Yueshu Li (1), Aleksandar Egi (1), Ken Sawatzky (1), Michael Edney (2), Marta Izydorczyk (2), and Brian Rosznagel (3)

(1) Canadian Malting Barley Technical Centre, Winnipeg, MB, Canada; (2) Canadian Grain Commission, Grain Research Laboratory, Winnipeg, MB, Canada; (3) Crop Development Centre, University of Saskatchewan, Saskatoon, SK, Canada

Brewing trials were carried out with malt made from Canadian hulless barley varieties, CDC Freedom, CDC McGwire, and CDC Gainer, generated from pilot malting trials at the Canadian Malting Barley Technical Centre. All-malt brews were done with 100, 90, and 70% hulless malt. Those brews done with 90 and 70% hulless malt were supplemented with 10 and 30% normal commercial malt. For all brews, the following standard procedure was used to facilitate the comparison. Malt was milled using a hammer mill. Mash in was at 48°C for 30 min. The temperature was increased to 65°C, held for 30 min, and finally raised to 76°C for mash off. Wort separation was carried out using a mash filter. The brews were boiled for 90 min, and hops were added at 90 and 5 min before the knockout, respectively. For all the brews, the finished wort was cooled to 12°C, pitched with a commercial lager yeast, and fermented at 15°C. The beer was aged for 7 days at -1.5°C, filtered using a cellulose acetate pad filter, carbonated, packaged into bottles, and pasteurized (10 pasteurization units). No difficulties were experienced during mashing for all three malt varieties. Conversion times for the trial hulless varieties were longer than for the control commercial malt. The control converted in 11 min, while the trial hulless varieties converted after 25 to 30 min. Conversion time in the trials was reduced as the proportion of commercial malt in the trials increased. Wort separation time from the mash filter was also significantly higher for the hulless trials, although it was found that there was no correlation between the beta-glucan content of the blend and the runoff time. CDC McGwire malt exhibited a faster runoff, followed by CDC Freedom and CDC Gainer. Under the same fermentation conditions, all brews fermented well and the achieved attenuation was comparable to that of the control brew with commercial malt. Beer foam stability was equal to or better than that of the control brew. Beer residual sugars of hulless brews were also comparable to those of the control brew. Physical stability of the beer produced from the trial varieties was poorer than that of the control, although there was variation among the varieties. Beer sensory results indicated that the quality of beer brewed from the hulless barleys was satisfactory and no quality defects were noticed.

Rob McCaig has more than 22 years of brewing industry experience with Molson Breweries. Starting his career in 1981 with Molson in Quebec, Rob has held a number of positions including research microbiologist, brewer, corporate brewer, and brewmaster. In February of 2003, he left Molson to take the position of managing director and director of brewing for the Canadian Malting Barley Technical Centre (CMBTC) in Winnipeg. Rob is a member of the American Society of Brewing Chemists (ASBC), serving as both local chairman and as president of the national ASBC. He is also a member of the Master Brewers Association of the Americas and the Institute and Guild of Brewing. While working as a research microbiologist, Rob presented and published more than 20 research papers. He has a M.Sc. degree in applied microbiology from the University of Guelph.

O-38

Withdrawn

O-39

Investigation into Conditions During the Early Stage of Kilning to Improve Beer Flavor Stability

TSUTOMU UEDA (1), Katsuya Sasaki (1), Hiroshi Itagaki (2), Kumiko Inomoto (1), Noboru Kagami (1), and Katsuya Kawatsura (1) (1) Asahi Breweries, Ltd., Brewing R&D Laboratory; (2) Asahi Beer Malt Co., Ltd.

A cardboardlike flavor is still noted as one of the stale flavors in aged beer, and it is well-known that *trans*-2-nonenal (T2N) is responsible for the cardboard flavor. While there were several studies of curing conditions in malting process for reducing the T2N level, there have been few investigations into the early kilning (withering) stage. Therefore, withering conditions were evaluated by using two indicators: lipoxygenase (LOX) activity and malt *trans*-2-nonenal potential (M-T2N-P), a recently found malt indicator of T2N formation in aged beer. In this study, we conducted malting trials with different parameters of humidity and temperature of inlet air and the airflow level in the withering process and compared the changes in LOX activity during the kilning process, LOX activity, and M-T2N-P in the finished malt. As a result, while each measure of reducing the temperature of inlet air and increasing the airflow level was observed to be effective, the measure of reducing the relative humidity of inlet air was considered as a most effective measure to decrease peak LOX generation in the kilning stage, LOX activity, and M-T2N-P in malt. Furthermore, the best practice in a commercial malting plant was performed with the above three withering measures combined with a previous measure of increasing the curing temperature. The optimization of these parameters enabled a considerable 70% reduction in LOX activity and a 30% reduction in M-T2N-P, although there was no particular difference in the other standard malt analyses. In addition, the significance of these measures was also confirmed in comparative brewing studies that evaluated beer flavor stability based on T2N reduction and sensory tests. Taken together, we concluded that kilning conditions are potentially important factors in reducing the T2N level in aged beer.

Tsutomu Ueda graduated from Osaka University in 1992, majoring in bioengineering. Since graduation he has worked in various positions for Asahi Breweries Ltd. From 1992 to 1995, he was assigned to the Mashing Section staff of the Fukushima Brewery. During this time, he worked on the development of the bottom-entry mashing-in system for improving beer flavor stability. That work was presented at the 26th EBC Congress (Maastricht, 1997). From 1995 to 1997, he worked as a malting supervisor at Asahi Beer Malt Ltd. In 1997 and 1998, he served as chief in the Quality Assurance Section. He spent 1999 as a visiting researcher at Brewing Research International (BRI) in the U.K. Upon returning to Japan, he was appointed malt specialist in the Brewing R&D Laboratory. His primary area of interest is malting technologies for improving beer flavor stability. He is currently malt specialist and assistant section manager in the Brewing R&D Lab of Asahi Breweries Ltd.

TECHNICAL SESSION VII: Flavor

Moderator: Sue Thompson

Suzanne Y. Thompson is sensory manager at Miller Brewing Company, Milwaukee, WI. She has 24 years of sensory experience in the brewing industry. At Miller, she is responsible for establishing and administering company-wide sensory programs that include descriptive panels, quality assurance panels, and consumer panels. Suzanne received a B.S. degree in food science from the University of Wisconsin-Madison in 1980. She is currently president of the American Society of Brewing Chemists (ASBC) and has been past president elect (2002-2003), vice president (2001-2002), ASBC Newsletter editor (2000-2001), and secretary (1996-1998) of the ASBC. She has been an active participant of several ASBC subcommittees, chaired the Difference-From-Control Sensory Test subcommittee in 1999, and lead several taste training sessions at the ASBC annual meeting. Suzanne is an experienced judge at the World Beer Cup and the Great American Beer Festival. She is also a member of the Institute of Food Technologists and American Society for Testing and Materials.

O-40

A Survey About Different Fractions of Hydroxy Fatty Acids During Malting and Brewing and Their Importance for Beer Flavor Stability

STEFAN MEYNA and Karl Wackerbauer

University of Technology of Berlin, Chair of Brewing Science; FBM der VLB Berlin

Hydroxy fatty acids have been known as indicators for oxidative reactions and aging processes in any kind of living organism (plants, animals, and even human beings) for many years. These lipid oxidation products are also found to be a precursor of flavor intensive carbonyls in foodstuffs and, therefore, are also of importance for beer flavor stability. Foam-negative reactions and a bitter flavor are further properties of such hydroxy fatty acids in beer. This study gives a survey about the different fractions (e.g., free and triglyceride-bonded components) and concentrations of these acids in barley, malt, wort, and final beer, measured by gas chromatography-mass spectrometry. Moreover, the influence of some technological parameters in the malt- and brewhouse on the hydroxy fatty acid concentration is shown, e.g., behavior during the whole malting process; influence of kilning temperature, milling, mashing-in temperature, and oxygen supply in the brewhouse; aeration during fermentation; etc. One essential result was that lipid oxidation already starts with the barley growing on the field due to defense mechanisms of the plant (activation of lipoxygenase pathway). A noticeable increase of, especially, trihydroxy fatty acids during 6 months of proper barley storage was also found. Thus, different barley varieties were also stored under extreme conditions (very high and low temperatures) and then malted to get detailed information about the influence of barley storage parameters on later beer flavor stability. From the produced malts, beers were brewed under constant conditions in a 1-hL pilot plant and the flavor stability of the beers was evaluated by different analytical and sensory methods. Additionally, seven common barley varieties from the 1999 and 2000 crops, stored for 3 resp. 4 years were also analyzed concerning their amount of hydroxy fatty acids and the results were compared with those measured in the fresh state and after the mentioned 6-month storage period. Furthermore, these barleys were malted and, from the malts, beers were produced and evaluated concerning flavor stability.

Stefan Meyna studied in the field of brewing science at the University of Technology of Berlin (1993–1999), graduating as an engineer (Dipl.-Ing.) in 1999. Since 1999, Stefan has done research work concerning beer flavor stability and lipid oxidation as a doctoral candidate (PhD) at the Chair of Brewing Science, University of Technology of Berlin and FBM der VLB Berlin (head: Prof. Dr.-Ing. Karl Wackerbauer). In 2000, Stefan became a research assistant with instructional work at the Chair of Brewing Science, and in 2001, he became head of the laboratories of the FBM.

O-41

Relationship Between the Flavor Compounds Formation and the Gene Expression Profiles of Brewing Yeast

ATSUSHI FUJITA (1), Nobuyuki Fukui (2), Hiroto Kondo (1), and Yasutsugu Kawasaki (1)

(1) Suntory Ltd., Institute for Beer & RTD Development; (2) Suntory Ltd., Process Development Department

The progress of DNA microarray technology has made overall analysis of gene expression possible; thereby, we are now able to obtain comprehensive information about the physiological state of the yeast during fermentation and to disclose the relationship between the manner of gene expression and the development of various flavor compounds. Although the microarray technology is currently based on the genomic sequence of the yeast *Saccharomyces cerevisiae*, S288C, we have already reported in ASBC 2003 held in Albuquerque that it could be applicable to the analysis for general brewing lager yeasts, *Saccharomyces pastorianus*. In this study, we conducted two distinct beer fermentations using all-malt and adjunct, 25% malt ratio, worts with the same brewing lager yeast. We investigated the fermentation performance, esters or fusel alcohols formation, and gene expression profiles of the yeast cells during the course of fermentation using a DNA microarray. We found that difference in nitrogen contents between all-malt and adjunct worts resulted in distinct gene expression profiles, fermentation performance, and flavor compounds formation. This enables us to identify the genes corresponding to the synthesis of various compounds and to obtain useful information on the physiological states of the yeast affecting the formation of flavor compounds during beer fermentation. Our study may guide the adjustment of the brewing process so as to realize our desired qualities and moderate aroma.

Atsushi Fujita is a researcher in the Institute for Beer & RTD Development of Suntory Ltd. The main subject of his work is the optimization and development of the fermentation process of beer. He majored in nutritional chemistry in Kyoto University and engaged in clarification of effects of nutritional compounds on the formation of adipose tissue that stores fat. He joined Suntory Ltd. in 1992. He moved to Kyoto Brewery after the first 6 years in the laboratory for research on yeast metabolism and its modification by genetic engineering. Since 2002, he is again in this laboratory and is now engaged in the development of fermentation for happou-shu that has poor nutritional compounds.

O-42

Withdrawn

O-43

Improvement of Beer Flavor Stability by Reducing Deterioration Precursors in Malt

TAKAKO INUI, Nobuo Tada, Norihiko Kageyama, Seisuke Takaoka, and Yasutsugu Kawasaki
Suntory Ltd.

Stale flavor substances of beer, exemplified by some kinds of aldehydes, are generated by various chemical reactions during the brewing process and storage. Some aldehydes are generated by enzymatic or nonenzymatic reactions of lipids and fatty acid and others by heat chemical reactions, where amino acids are added to amino-carbonyl compounds or ox-phenolic compounds. We investigated the influence of these deterioration precursors in malt on flavor stability. It was found that beer flavor stability was improved by reducing deterioration precursors in malt. Based on these findings, we developed our novel method that removed the lipid, fatty acid, amino acid, and polyphenol adequately from malt. As a result, we succeeded in vastly improving beer flavor and flavor stability.

Takako Inui graduated from Kyusyu University. She began employment with Suntory Ltd. in 1989 as a researcher in the Institute for Fundamental Research. Since 2002, she has worked at the Institute for Beer Development. She has been studying the development of brewing technology.

O-44

Sensory Techniques for Understanding Consumer Preference

DEBBIE PARKER and Sarah Norman
Brewing Research International

In today's highly competitive environment, it is becoming increasingly difficult for companies to maintain a competitive advantage. It is no longer simply a case of producing a good product—the whole marketing mix has to be right and consistent product quality has to be delivered. Recent studies at BRi show that the mix is often not right, with drinkers being frequently impressed by a drink proposition but then being disappointed by the taste of the product. One of the reasons for this is that many product development decisions are made primarily on the basis of traditional consumer research techniques. However, these techniques have a number of inherent faults. Market research techniques are excellent tools for establishing what consumers like, but they are not very reliable when it comes to understanding why consumers prefer one product to another. This is due to either consumers not being able to find the right words to describe why they like something or because they use the wrong words, which can be misleading to those interpreting the results. Sometimes consumers simply do not know why they prefer one product to another. This problem can be overcome by using a combination of trained sensory panels and consumer research. Unlike consumers, trained tasting panels operate objectively and can accurately describe the sensory attributes present in a range of beverages and the extent to which each influences the overall characteristics of an individual product. Recently, advances have been made, employing multivariate analysis, that involve combining sensory and market research techniques. In this way, it is now possible to understand the underlying sensory characteristics that are driving consumer preference. Furthermore, analytical studies can measure the balance of key flavor compounds that contribute to the overall flavor and can be mapped together with sensory and market research data. These chemical analyses can provide the information necessary to change sensory attributes of a product in the certain knowledge that these changes

will increase consumer acceptance. This unique approach to consumer research provides actionable and reliable diagnostic information that companies can use for a product's formulation, packaging, and brand positioning. This paper describes work using these techniques and presents consumer, sensory, and analytical data for different beverage categories and provides an insight into the individual flavor notes that drive consumer acceptance.

Debbie Parker joined BRI in 1988 with an Honours degree in biochemistry, passed the Institute of Brewing Associate Member Examination in 1991 and has recently been awarded a doctorate in brewing science. She is a frequent lecturer at industry training courses and technical meetings and is an experienced taster. Debbie is also a member of the EBC Sensory Subgroup. An accredited trainer (City and Guilds 7307), Debbie now designs and delivers sensory training courses and workshops. A professional beer taster for 13 years, Debbie has applied her tasting skills as a judge at competitions such as 'The Beauty of Hops' and the Great British Beer Festival.

O-45

Unraveling Beer Flavor Through the Use of Gas Chromatography-Olfactometry

Alicia Carruthers, Meaghan Culbert, Michel Libon, DAVID MARADYN, Mick McGarrity, Jerome Pellaud, Robert J. Stewart, and Don Thompson
Global Innovation and Development, Interbrew

Flavor is arguably the most important attribute of beer, but one of the least understood. A greater understanding of beer flavor, particularly what individual chemical compounds are responsible, their origins and concentrations would be of great benefit to the brewer. This knowledge could lead to greater product quality and consistency, reduce the incidence of flavor defects, and allow manipulation of beer flavor through ingredient selection and/or process changes. Identification of chemical compounds important to flavor is a difficult process. These compounds are typically present in extremely low concentrations and can be reactive or fragile with respect to sampling techniques. Separation by gas chromatograph is complicated since non-flavor-active compounds overshadow those of interest. The detector of choice for flavor research is the human nose, with the ability to detect only those compounds important to flavor and also differentiate each flavor-active compound in terms of its odor descriptor and intensity in the matrix. This technique is termed gas chromatography-olfactometry (GCO). It has enjoyed widespread use by those investigating the flavor of fruit, juices, wine, and spirits, but reports of its use in the brewing industry is limited. This paper will illustrate how we have used the GCO technique in profiling two of our lager beers and briefly investigated the effect of cold aging on the flavor. Aroma extract concentration analysis (AECA) was performed on two lager beers (A and B) at five different flavor dilutions and analyzed by GCO to identify and rank important flavor clusters in each. Sixty-three flavor clusters were identified in lager A and 54 flavor clusters were identified in lager B. Flavor cluster identification was accomplished by GC/MS spectral matching, relative retention index (RRI), and odor descriptor comparison to literature values. Forty-five of the flavor clusters in lager A and 43 of the flavor clusters in lager B were identified to a characteristic flavor compound. Although there were many flavor clusters common to both lager A and lager B, both possessed distinctive flavor clusters that contributed to their respective flavor. GCO was used to look for flavor differences between fresh, 9- and 12-month cold-stored lager B. Three flavor dilutions of each product were evaluated by the GCO panel. It was found that the fresh and cold-stored lager differed significantly; fruity, estery, and floral flavor clusters present in the fresh product were of less importance to the overall beer flavor, while smoky, cheesy, rubbery, and malty flavor descriptors increased in importance. Our research demonstrates that the GCO technique can be a valuable tool for the brewer interested in investigating beer flavor and flavor stability.

David Maradyn received a B.Sc. degree in chemistry in 1991 and a Ph.D. degree in organic chemistry in 1996 from the University of Western Ontario, London, Ontario, Canada. He joined the Labatt Brewing Company as a postdoctoral fellow in 1995, working in the Advanced Development department. Since October 1997, he has been working as a research scientist with the Global Innovation and Development department of Interbrew. David has served the ASBC as member and chair of technical subcommittees and is currently a member of the Technical Committee.

O-46

Actual Aspects of the Analytical Prediction of Flavor Stability

OLIVER FRANZ and Werner Back

Lehrstuhl für Technologie der Brauerei I, Technische Universität München, Freising, Germany

A very important field of research is the analytical prediction of flavor stability. Many analytics have been introduced, such as the Lag-Time, which can be measured by electron spin resonance-spectrometry; the determination of flavor compounds (aging indicators) by gas chromatography, which increase significantly during aging; the content of sulfur dioxide in beer, produced by the yeast; the polymerization index, related to the content of phenolic substances, which represents the oxidative stress during mashing; the reducing power, which is related to melanoidins; and finally, the sensory test, which is used as a reference for all analytical systems but is sometimes not seen as an analysis by itself, which you have to take care of in the same way. It has to be considered what kind of aging test you are using: natural aging or a forced aging test, which is important for the prediction. From experience, we know that people have difficulties in evaluating and judging the results of the tests. The question is whether only one test is enough to determine flavor stability. Breweries often have problems in a methodical approach to technological problems. Flavor stability is often seen as a luxurious product and not as routine quality control. But it should be the consequence of a high level technology. So the whole brewing process should be considered and a step control should be established. There are published methods to determine free radicals in malt by ESR. It could be shown that the amount of free radicals depends on the variety and the provenience. The content of phenolic substances did show a relationship as well. With a chemiluminescence detector, oxidation reactions during mashing could be detected and specific behaviors of different malts could be evaluated. These analytics could be new quality criteria for malt with regard to flavor stability. Disinfection agents, based on hydrogen peroxide, could decrease the flavor stability clearly, if the tanks are rinsed inadequately. This effect can be seen by the Lag-Time measurement. Ferrous ions from filter aids can also influence the Lag-Time and flavor stability negatively. The spectrum of phenolic substances is clearly modified by PVPP treatment. Within a brewery, using a constant dosage of PVPP, tannoids can be a good indicator for flavor stability. A comparison of a forced aging test and natural aging did show a discrepancy in the analytical results of the aging indicators. During natural aging, you can see the impact of the migration of oxygen into the bottle (depending on the quality of the crown cap). To specify the flavor stability of the fresh product beer, a forced aging test is useful. You can also detect technological improvements easily.

Oliver Franz studied brewing and beverage technology at the Technical University of Munich-Weihenstephan and graduated September 1998. Since February 1999, he is working on his doctoral thesis at the Chair for Brewing Technology I (Prof. Back) in Weihenstephan. His theme was "Systematic investigations on the endogenous antioxidative activity of beer in consideration of technological features". Since May 2000, he has been working as the head of the laboratory for GC/HPLC-Analytics at the Chair for Brewing Technology I in Weihenstephan. His areas of research are the impact of raw materials and brewing technology on beer flavor and flavor stability, analytical methods to determine flavor stability, and antioxidant activity of beer and its optimization during the brewing process.

TECHNICAL SESSION VIII: Analysis

Moderator: Jean-Pierre Dufour

Prof. Dr. Ir. Jean-Pierre Dufour has received M.Sc. (1975) and Ph.D. (1979) degrees (Louvain). Jean-Pierre was a research fellow at Johns Hopkins University, School of Medicine, Baltimore, MD, from 1979 to 1981. Jean-Pierre has been professor and head of the Department of Brewery and Food Industries, Catholic University of Louvain (1981–1993); visiting professor, Escola Superior de Biotechnologia (Porto, Portugal) (1989–1994); associated professor, University Senghor (Alexandria, Egypt) (1992–1995); expert for EEC and UNIDO (1994–1996); and professor (1995–present) and chair and head of the Department of Food Science, University of Otago, Dunedin, New Zealand. Jean-Pierre's expertise is in flavor science, fermentation science and technology, malting and brewing sciences, and yeast

biochemistry/enzymology. Jean-Pierre is a member of EBC Brewing Science Group, ASBC, IOB, IFT, and ACS. Jean-Pierre is president and fellow of the NZIFST and the New Zealand delegate to IUFoST.

O-47

The Enrichment of Foam-Positive Substances by the Use of Ultrafiltration

DENIZ BILGE, Karl Wackerbauer, and Marc Rauschmann
University of Technology of Berlin, Chair of Brewing Science; FBM der VLB Berlin

Molecules in liquids can be separated according to their molecular size by the application of ultrafiltration. A couple of test series were carried out with a pilot plant using membranes with effective cut-offs in the range of 10 to 300 kDa in order to evaluate the application of this filtration technique. In practice, ultrafiltration could be used for the enrichment of high molecular substances in beer in order to improve head retention. It could be shown that this is an appropriate measurement to increase head retention of beer. The degree of the improvement of head retention depends basically on the cut-offs and the concentration rates. The performance of the filter in terms of flux in L/m²/h is mainly influenced by temperature, transmembrane pressure, cut-off, and the chemical composition of the beer. Analyses of the retentates and permeates confirmed that mainly high-molecular protein fractions, beta-glucans, polyphenols, and anthocyanogens are held back by the membranes and remain in the retentate. Color and viscosity are likewise increased. Permeates, as well, show lower values and reduced amounts of the according substances. Due to the enrichment of proteins and polyphenols in the retentates, the shear forces in the unit turbidity is increased and nonbiological stability is considerably reduced. By filtration and prestabilization, turbidity could be decreased and stability improved to the level of the initial beer.

Deniz Bilge attained his degree as graduate engineer for brewing technology at the University of Technology of Berlin. He began employment in November 1999 at the Chair of Brewing Science of the above-mentioned University as scientific assistant to Prof. Wackerbauer. Areas of activity are the supervision of official research projects in the institute and of students working on their diploma thesis. Among other things, he has worked on research projects dealing with flavor stability and cross-flow microfiltration. Currently, he is working on his Ph.D. degree about the application of ultrafiltration to improve head retention of beer.

O-48

The Relative Significance of Physics and Chemistry for Beer Foam Excellence

CHARLES W. BAMFORTH
University of California, Davis

The foaming of beer can be considered from the perspective of both physics and chemistry. In terms of physics, we may invoke phenomena such as nucleation, drainage, bubble size distribution, and disproportionation. From the aspect of chemistry, emphasis is placed on the relative proportions of foam-stabilizing and foam-destabilizing surface-active molecules. Seldom, if ever, have the two halves of the foam scenario been discussed together in the context of their relative significance. This paper presents a theoretical consideration of the interaction between the physics and the chemistry of foaming, informed by the myriad of studies that have been performed on beer foam in this and other laboratories. It looks, inter alia, at the relationships between bubble size, bubble wall thickness, molecular dimensions of foaming polypeptides and other surface-active species, kinetics of intermolecular interactions, and rates of gas diffusion.

Charlie Bamforth became the first Anheuser-Busch Endowed Professor of Malting and Brewing Sciences at the University of California, Davis in February 1999. He has more than 25 years of experience in the brewing industry, previously holding senior positions with Brewing Research International and Bass. Charlie was the founding chairman of the European Brewery Convention Foam Sub-Group. A fellow of the Institute of Brewing and fellow of the Institute of Biology, he is editor-in-chief of the Journal of the ASBC. His book, Standards of Brewing, was published in 2003, together with the second edition of Beer: Tap into the Art and Science of Brewing. His latest book, Beer: Health and Nutrition, will be

released in 2004. He has also published books on biotechnology and soccer goalkeepers.

O-49

Comparison of Methods for Assessing Protein in Beer

KARL J. SIEBERT and P. Y. Lynn
Department of Food Science & Technology, Cornell University, Geneva, NY

There are numerous methods for determining proteins in beer. Many of these suffer biases and give differing responses depending on the nature of the protein or polyphenol molecules in a sample. Several important classes of beer proteins have been shown to have quite different amino acid compositions. These differences determine both the functional properties of the proteins and their responses in a number of analytical methods. The beer haze-active (HA) proteins are rich in proline and glutamine and respond very poorly in the Bradford method (Coomassie brilliant blue dye binding, CBB). Beer foam-active (FA) proteins, on the other hand, are rich in basic and aromatic amino acids and respond well to CBB. Conversely, haze induction upon addition of tannic acid (TA) responds well to HA protein and poorly to FA protein. HA polyphenols have at least two sites that can attach to HA proteins and, thus, bridge them together. Some of these bind more strongly to proteins than do other polyphenols and have been demonstrated to be more haze active. Some polyphenols have only a single binding site; they can attach to HA protein but do not lead to haze. Beer samples contain differing amounts of HA and non-HA protein, as well as HA and non-HA polyphenols. It was of interest to systematically compare the responses of a number of protein determination approaches on samples containing combinations of HA and non-HA proteins and HA and non-HA polyphenols. In a preliminary study, water-soluble gliadin and methyl gallate were used as surrogates for beer HA protein and non-HA polyphenol, respectively. Development of a procedure to isolate water-soluble hordein from malt and the use of epicatechin, a beer polyphenol that has been shown to be non-HA at modest temperatures, provide more realistic alternatives. Mixtures containing various combinations of HA and non-HA proteins (water-soluble hordein and lysozyme, respectively) and HA and non-HA polyphenols (tannic acid and epicatechin, respectively) were prepared in buffer model systems. A battery of protein methods (the Bradford method, TA haze induction, the Bicinchoninic acid (BCA) method, and 280 nm absorbance) were applied to each sample and the results were compared. The various methods gave quite different responses to the different test compounds. With concentrations of the various substances roughly equivalent to those found in beer, 280 nm absorbance and BCA suffered strong interference from the polyphenols. The CBB and HA protein methods, on the other hand, responded very little to polyphenols. CBB response was much greater to lysozyme than to hordein. TA haze induction responded only to HA protein, but exhibited some nonlinearity. Results obtained when these methods are applied to beer must be interpreted with caution.

Karl Siebert received a Ph.D. degree in biochemistry from Penn State in 1970. He joined the Stroh Brewery Company in Detroit, where he spent 18 years and held positions from research associate to director of research. In 1990, Dr. Siebert joined Cornell University as professor of biochemistry in the Department of Food Science and Technology. He served 5 years as department chairman and now has a predominantly research appointment. Dr. Siebert served on ASBC technical subcommittees and was a member and chairman of the Technical Committee. He is serving his second stint on the Journal of the ASBC editorial board (1980–1992; 1996–present). He is active as a consultant in the beverage industry.

O-50

Beer Foam Stability—The Role of Specific Polypeptides

GRAHAM G. STEWART (1), Stephan Brey (1), James H. Bryce (1), Samodh de Costa (1), Kenneth Leiper (1), Wilfrid J. Mitchell (1), and Ian McKeown (2)

(1) The International Centre for Brewing and Distilling, Heriot-Watt University, Edinburgh, Scotland; (2) Ineos Silicas, Warrington, England

The ability of beer to produce a stable foam is an important feature in the attractiveness of the product to the consumer. Beer foam is affected by many factors, such as lipids, iso-alpha-acids, metal ions, melanoidins, polyphenols, ethanol, and polypeptides. The structure of beer foam is complex, with a network of hexagonal bubbles, the walls of which

comprise surface-active compounds. Primarily, polypeptides associate with carbohydrates, metal ions, and iso- α -acids to form a network of bubbles, giving a stable head of foam. Beer polypeptides (small proteins) are a mixture of complicated proteinaceous substances, several of which are associated with foam stability. Studies in this laboratory have focussed on beer foam stability from three aspects. • The effect of silica gel. • The negative effect of high-gravity brewing and fermentation on foam stability. The fate of foam inducing polypeptides during this process. • Isolation, cloning, and characterization of beer foam polypeptides. The studies confirm that the major polypeptides associated with beer foam formation and stability are hydrophobic polypeptides. Specifically, they are lipid transfer protein (LTPI), a 17-kDa polypeptide, and protein Z, a 40-kDa molecule.

Graham Stewart received B.Sc. degrees in microbiology and biochemistry from the University of Wales and Ph.D. and D.Sc. degrees from the University of Bath. He was a lecturer in biochemistry at Portsmouth University from 1967 to 1969. He began employment with the Labatt Brewing Company in 1969, based in London, Ontario, Canada. He held a number of scientific/technical positions with Labatt's, and from 1986 to 1994, held the post of technical director. From 1994 to the present, he has been professor and director of the International Centre for Brewing and Distilling at Heriot-Watt University in Edinburgh, Scotland. He is a member of the Master Brewers Association of the Americas, the American Society of Brewing Chemists, the Institute of Brewing Studies, and the Institute and Guild of Brewing and, in 1999 and 2000, was this Institute's president.

O-51

Comprehensive Quality Assurance of Asahi Breweries, Ltd.

HISANORI OKITA (1), Yutaka Miyamoto (1), Hidetoshi Tezuka (1), and Yoshifumi Nishino (2)

(1) Quality Control Center, Asahi Breweries, Ltd.; (2) Production Headquarters, Asahi Breweries, Ltd.

The Asahi Breweries Group manufactures a wide variety of products, including beer, whisky, wine, liqueur, and *shochu* 'Japanese white liquor'. We also import beverages from abroad. Customer satisfaction is our primary concern. The following quality assurance system is adopted to rigorously guarantee the safety and quality of our products. 1) High-quality raw materials. To acquire high-quality raw materials, Asahi performs acceptance inspections based on our specifications, cross-checks analyses with suppliers, sampling inspections on particular items, and strict evaluation of our suppliers. In our evaluation of suppliers, we examine the compliance with our quality standards, inspect their plants, and recommend the required improvement to suppliers with low evaluations. 2) Effective monitoring of production processes. Every day we collect process-control index and analyze data obtained from every step of production processes. These efforts are to make sure the processes comply with our standards in every detail. The sensory test, one of the most important inspections, is conducted by in-house specialists. This test includes the daily evaluation held at each brewery and the regular corporate-wide test on products from all the breweries. The latter aims for standardizing and improving flavor characteristics of products manufactured by our breweries. 3) Swift distribution process through freshness management. We significantly reduced the time for distribution to satisfy the demand for freshness by Japanese customers. As a result, the sales volume has increased. Our efforts to further minimize the distribution time are currently in progress. 4) High-quality domestic and imported products. To assure safety and quality, we perform routine analyses of raw materials and products based on criteria specified by the regulations. As for imported products, we perform product analyses prior to the decision on marketing, conclude a quality agreement with manufacturers, and conduct acceptance inspections and routine analyses. We only purchase from the manufacturers that fulfill Asahi's quality standards. If an issue arises, corrective measures are discussed with manufacturers for solutions to the problem.

Hisanori Okita received a B.S. degree in fermentation engineering from Hiroshima University in Japan in 1983. He joined Asahi Breweries, Ltd. in April 1983. He has served as brewmaster in the Hokkaido Brewery, Suita Brewery for 11 years. He worked at Bass Brewing in 1990. He has also functioned as trainer in the Technical Training Center (1996–1999). Now he serves as deputy manager in the Quality Control Center (2002–present). He works to assure the quality of alcoholic beverages.

O-52

Application of a GC/MS Method Using SPE Columns for Quantitative Determination of Diacetyl and 2,3-Pentanedione During Beer Fermentation

JELENA PEJIN, Olga Grujic, and Sinisa Markov
Faculty of Technology, University of Novi Sad, Novi Sad, Serbia and Montenegro

Diacetyl and 2,3-pentanedione are important contributors to beer flavor and aroma. A new GC/MS method for the determination of diacetyl and 2,3-pentanedione was developed. The GC/MS method has good sensitivity and is currently the most accurate method available. Diacetyl and 2,3-pentanedione were derivatized with 1,2-diaminobenzene to form 2,3-dimethylquinoxaline and 2-ethyl-3-methylquinoxaline, respectively. The amounts of formed 2,3-dimethylquinoxaline and 2-ethyl-3-methylquinoxaline were proportional to the concentrations of diacetyl and 2,3-pentanedione present in the sample. 2,3-Dimethylquinoxaline and 2-ethyl-3-methylquinoxaline were extracted by solid-phase extraction (SPE) columns and determined by gas chromatography using a mass selective detector. Extraction by SPE columns proved to be very rapid, simple, and precise. This method can be used for simultaneous determination of diacetyl and 2,3-pentanedione concentrations in beer in a great number of samples. This method was applied for the determination of diacetyl and 2,3-pentanedione concentrations during beer fermentation (primary fermentation and maturation). During fermentation, diacetyl and 2,3-pentanedione were quantified to demonstrate the suitability of the method. Primary fermentations were carried out at different temperatures (8 and 14°C) and an industrial bottom-fermented yeast strain, *Saccharomyces uvarum* (*carlsbergensis*) was used. The aim of this investigation was to determine the influence of primary fermentation temperature and wort composition on diacetyl and 2,3-pentanedione concentrations. Corn grits, beside malt, was used for wort production. Level of corn grits varied from 10 to 40%. Diacetyl and 2,3-pentanedione formation and reduction were strongly influenced by temperature, and the rates for both increased with the increase of primary fermentation temperature. The highest diacetyl and 2,3-pentanedione concentrations (0.6365 and 0.8192 mg/L, respectively) were obtained during fermentation of wort with 40% of corn grits, at 14°C. This accurate determination of diacetyl and 2,3-pentanedione was a valuable tool for analyzing the influence of wort composition or fermentation conditions, such as primary fermentation temperature, on their formation and reduction. It is also well suited for the quality control of beer during fermentation.

Jelena D. Pejin was born in 1975 and has been employed as a teaching assistant for the course of Malt and Beer Technology at the Faculty of Technology, University of Novi Sad, since September 1999. Jelena graduated at the Faculty of Technology, Department for Microbiological Processes, in June 1999. At the beginning of the academic term for 1999/2000, she enrolled in postgraduate studies at the Microbiological Processes course. She received an M.Sc. degree for the field of beer fermentation in December 2003. Her professional work has included engagement in practical lessons for the course of Malt and Beer Technology and for B.Sc. papers for the same course. Parallel to the teaching process, Jelena has been included in the scientific work, and the results of these investigations have been already presented at domestic and international scientific and professional meetings and published in the respective proceedings as well as in domestic journals.

O-53

Beverage Appearance and Flavor Protection from Carbon Dioxide Quality Excursions

CHRIS DUFFELL and Robert Scrafton
domnick hunter Ltd.

Carbon dioxide is used in the beverage industry for brewing, carbonation, packaging, and dispense. In recent years, there has been increased awareness to the importance of carbon dioxide (CO₂) quality and its effects on beverage products. Quality guidelines for CO₂ used in the beverage industry are published by bodies such as the International Society of Beverage Technologists (ISBT) and European Industrial Gas Association (EIGA). These guidelines are intended to offer protection against naturally occurring CO₂ contaminants that could result in flavor defects of the beverage. Contaminants may also affect the appearance of the foam head and, hence, presentation of the beer. In addition,

contaminants are controlled by regulation for the prevention of physiological detriment. Detailed in this paper are measures that may be applied from plant-level protection, including HACCP, to retail dispense. In the event of a CO₂ quality incident, these measures will ensure beverage quality and freshness for the consumer. In-line purifiers have been designed that act as a final multilayer barrier filter against trace contaminants in a CO₂ gas supply. The performance of these purifiers was evaluated by introducing contamination to beverage-grade CO₂ to provide an inlet challenge in excess of the ISBT specification. The data presented show that removal of typical contaminants, including aromatic hydrocarbons and sulfur-containing compounds, can be achieved. This multilayer barrier filter technology is currently being utilized globally at a plant level and, more recently, has been applied to offer the same benefits to draught beer dispense outlets.

Chris Duffell received an M.Phys. in astrophysics from Cardiff University in 1997. He then began employment at the National Engineering Laboratory in Scotland as a project engineer in the oil and gas laboratories in Flow Centre. Chris achieved chartered physicist status in 2000. He then joined the Postgraduate Training Partnership scheme between NEL and Strathclyde University. His Ph.D. project was entitled "The optimisation of ultrasonic flowmeter design". Chris presented details of his work in four technical papers at international conferences. Upon completion of this scheme, Chris began employment with domnick hunter ltd. as senior development engineer in Newcastle. His work now involves product verification and development as well as the production of technical literature.

TECHNICAL SESSION IX: Microbiology

Moderator: Candace Wallin

Candace E. Wallin is an instructor for the brewing science and technology courses offered through University Extension, University of California, Davis. She also manages the brewing laboratory and pilot brewery at UC Davis. She has more than 25 years of experience in the brewing industry, including 18 years at Miller Brewing Company, Milwaukee, WI, where she worked initially as a microbiologist and then as a development brewer. She also gained 4 years of experience in the microbrewery segment of the industry while working as the microbiologist for Sudwerk Privatbrauerei Hubsch in Davis, CA. Candace is an associate member of the Institute & Guild of Brewing, as well as a member of the American Society of Brewing Chemists and the Master Brewers Association of the Americas.

O-54

Microbial Attachment and Biofilm Formation in Brewery Bottling Plants

ERNA STORGÅRDS (1), Kaisa Tapani (2), Peter Hartwall (3), Riitta Saleva (4), and Maija-Liisa Suihko (1)
(1) VTT Biotechnology, Finland; (2) Oy Sinebrychoff Ab, Finland; (3) Oy Hartwall Ab, Finland; (4) Olvi Oyj, Finland

Microbiological risk management is essential in the production of high-quality beer since quality defects may lead to substantial economic losses. The hygiene of process surfaces essentially affects the quality of the final product. The brewing industry is prone to biofilm formation due to the abundance of water needed at every stage of production. Biofilms develop when attached microorganisms secrete extracellular polymers, which in turn protect them effectively against cleaning and sanitation. In the current study, sterile stainless steel coupons were mounted onto critical sites of the fillers in three brewery bottling plants. Microbiological samples were taken from the coupons to reveal the pioneer organisms in biofilm formation. Microbiological samples were also taken from different horizontal and vertical surfaces close to the open product at the filler and crowner in order to be able to compare the microflora on the coupons with process surfaces in use. The pioneer bacteria were identified by ribotyping, carbohydrate fermentation tests, and partial DNA sequencing. The effect of sugars and sweeteners on attachment of pioneer organisms to stainless steel was studied in 1 mM phosphate buffer and analyzed by epifluorescence microscopy. The biofilm formation rate was studied for 8 weeks by successively dislodging the test coupons from each sampling site and examining them by epifluorescence microscopy. The results showed that pioneer microbes accumulated on new stainless steel surfaces within hours after the start of production. Regular daily cleaning reduced the number of microorganisms on the surfaces only momentarily. Canning

machines were markedly less prone to accumulation of microorganisms than bottling machines. Gram-negative bacteria, yeasts, and molds were the first to colonize the surfaces. Attachment of pioneer species to stainless steel was increased substantially by sugars and, surprisingly, also by intense sweeteners. Horizontal surfaces were prone to microbial accumulation and should be avoided in constructions as much as possible. Furthermore, biofilm formation occurred on certain surfaces despite daily cleaning and disinfection.

Erna Storgårds holds a first degree and a Ph.D. degree in microbiology from Helsinki University. She began employment with VTT Biotechnology in May 1988 as a research scientist in the microbiology department. Since January 2002, she has been group manager of the Microbial Diagnostics and Taxonomy research group. Her special field of expertise is process hygiene in beer production and dispensing. During her time at VTT, she has coordinated and participated in several national and international research projects, as well as been responsible for carrying out confidential contract research for industrial partners in Finland and abroad. Her current activities include research on biofilms and surface-microbe interactions. She has been a member of the EBC Microbiology Group, later the EBC Brewing Science Group, since 1992 and its vice chair since 2001; chair of the EBC Microbial Contaminants Subgroup since 1993; and member of the EBC Analysis Committee Microbiology Subcommittee since 1998.

O-55

Molecular Methods for Detection and Identification of Microbial Contaminants in Brewing Quality Control

AULI HAIKARA, Riikka Juvonen, Maija-Liisa Suihko, Teija Koivula, and Erna Storgårds
VTT Biotechnology, Finland

Current cultivation-based methods used by most breweries for microbiological quality control reveal possible contamination only after several days or weeks of delay. Moreover, they do not discriminate between spoilage and nonspoilage microbes or allow the detection of viable but noncultivable cells or tracing of contamination sources. In recent years, several promising molecular biological applications have been described for the detection, characterization, and identification of brewery contaminants. Group-, genus-, and species-specific PCR tests have been designed and evaluated for brewery contaminants, i.e., for lactic and acetic acid bacteria (*Lactobacillus*, *Pediococcus*), strictly anaerobic bacteria (*Pectinatus*, *Megasphaera*, *Selenomonas*, *Zymophilus*), enterobacteria, and wild yeasts. Various PCR detection formats (PCR-ELISA, LightCycler™ PCR, standard PCR) have been set up for most of these organisms. In order to detect the very low amounts of microbes in brewing samples, an enrichment method has been devised. Practical pre-PCR treatment methods, including collection of cells, DNA extraction, and removal of PCR inhibitors, have been developed for filterable (such as bright beer and process water) and nonfilterable (pitching yeast, wort, fermenting wort) samples. Modular PCR kits for standard PCR and real-time PCR have also become available from several companies. All developed PCR applications are, despite the enrichment step, more rapid and specific than the cultivation methods. Currently, PCR without prior cultivation is less sensitive and more expensive to use than cultivation. Denaturing gradient gel electrophoresis (DGGE) for separation of bacterial or eukaryotic ribosomal DNA amplicons is a valuable tool for characterizing microbial communities in specific environmental niches. This technique has also been applied to study and compare microbial communities during beer, wine, and whisky production. The automated ribotyping system (RiboPrinter® System DuPont Qualicon, U.S.A.) is a rapid molecular biological method for the identification and characterization of bacteria to species or even below species level. A comprehensive identification database has been created for brewery contaminants, such as *Lactobacillus* spp., *Pediococcus* spp., *Pectinatus* spp., *M. cerevisiae*, and *Obesumbacterium proteus*, using three different restriction enzymes. In addition to identification and renaming of bacteria, the database has been applied to tracing of contamination sources and detection of troublesome house flora in industrial processes.

Auli Haikara is chief research scientist at VTT (Technical Research Centre of Finland) Biotechnology in the research field of microbiological safety. She graduated in 1965 from the University of Helsinki with an M.Sc. degree in nutrition chemistry. In 1984, she received a Ph.D. degree in microbiology from the University of Helsinki. Since 1993, she has been

a docent in industrial microbiology in the Department of Applied Chemistry and Microbiology of the University of Helsinki. Her areas of research have included cereal microbiology focusing on gushing, active and toxigenic fungi, anaerobic beer-spoilage organisms, rapid detection methods, and antimicrobials produced by lactic acid bacteria. She has coordinated an EU-funded project developing PCR technology for use in breweries and has participated in an EU project on prevention of ochratoxin A in cereals. She is a member of the EBC Brewing Science group, chair of the Gushing subgroup, and a member of the Microbial Contaminants subgroup. She is also a member of ASBC.

O-56

Microsieves and Fluorochromes—A New Application to Detect Beer-Spoiling Microorganisms

KARL-JOSEF HUTTER (1), Dieter Kemenji (2), Frank Nitzsche (3), and Britta Kuhmann (1)

(1) Eichbaum Brauereien AG, Mannheim, Germany; (2) Schleicher & Schuell, Dassel, Germany; (3) Koenig Brauerei, Duisburg, Germany

Indirect membrane filtration followed by incubation on selective artificial media at 28–30°C for detection of beer-spoiling microorganisms is routinely used in brewing laboratories, although this procedure is very time consuming. Microorganisms that are able to form a colony on selective media were counted (CFU). Contaminants that are in their quiescent growth phase, senescent cells, and membrane-damaged cells do not form colonies on selective media during the short investigation time of 2–3 days. Furthermore, only those contaminants form colonies that are able to metabolize the artificial media. We have developed a direct and rapid method to count all viable (quiescent and not culturable cells) and dead contaminants without pre-enrichment on artificial media. Instead of membrane filters, we used microsieves. This new filter material consists of silicone strings with a mesh size of 0.45 µm. The contaminants were stained by different dyes, fluorogenic substrates, or dye combinations. Yeast cells were stained simultaneously with Fluorescein Diacetate (FDA) and Propidium Iodide (PI), while bacterial contaminants were fluorochromized with BacLight. Beside these dye combinations, we employed other stains like Sytox orange, Sytox orange in combination with FDA, Sytox green, Oxonol, and Berberine. In order to identify important beer-spoiling contaminants, we raised antibodies against *Lactobacillus* and *Pediococcus*. This rapid method to assess total counts and viability of beer-spoiling microorganisms will improve the microbiological quality control in the brewing industry. Furthermore, this method could also be applied to determine the health and viability of brewing yeast and hence improve fermentation performance and yeast handling procedures.

Karl-Josef Hutter was born in 1943 in Dietfurt/Altmühl, Bavaria. Karl-Josef's native country is the Federal Republic of Germany. Karl-Josef had vocational training at Brauerei Frankenheim, Düsseldorf (1960–1962), and studied brewery technology at the TU-Berlin (VLB) (1965–1970) and graduated from there in 1974. Karl-Josef has worked as a scientist at Fraunhofer-Gesellschaft (1970–1979) and as a scientist at the German Cancer Research Center, Heidelberg (1979–present). Karl-Josef has had lectureships at the University of Heidelberg (1985–1992), the University of Hohenheim (1994–1999), the Fachhochschule Mannheim (1995–present), and the TU-Dresden (1999–present).

O-57

Microbiological Quality Control in Breweries Based on Real-Time PCR—Implementation Experiences and Future Potential for Brewery Application

ANDREAS BRANDL and Eberhard Geiger

Technische Universität München, Center of Life Sciences Weihenstephan, Chair for Brewing Technology II, Germany

Common routine brewery quality control methods based on cultivation do not allow proactive process control because possible contaminations will often be detected after several days or weeks of delay. Contrary to these methods, real-time PCR assays have been developed that allow rapid and specific detection of low levels of microbes. Various real-time PCR formats (LightCycler™, TaqMan) are available now for group- or species-specific detection of virtually all beer-spoilage bacteria (*Lactobacillus* spp., *Pediococcus* spp., *Megasphaera* spp., *Pectinatus* spp.). During the last 2 years, the implementation of the method into laboratory routine was evaluated in several European breweries. Process samples were examined

with PCR and established in-house methods in parallel. The achieved results were recorded and extensive data on practical PCR performance was obtained. In the context of the brewery trials, PCR assays were improved for bright beer and yeast-containing process samples and pre-PCR steps, such as pre-enrichment and DNA isolation, were optimized in respect of analysis time, user-friendliness, and sensitivity. Both real-time PCR and standard PCR were tested in the breweries, but real-time PCR was shown to be the preferred method. Using PCR, it was possible to achieve results faster and more exact than with the conventional methods. Especially for troubleshooting purposes and the tracing of infection sites, PCR has been proven to be a very helpful tool. Nevertheless, it is recommended to use the method in combination with a short pre-PCR enrichment step to achieve the sensitivity required and to ensure that the detected cells are viable. Using PCR without pre-enrichment causes higher costs due to more laborious pre-PCR sample preparation steps. Based on these results, several developments are ongoing to make further use of the potential of PCR. A promising approach to differentiate between viable and dead cells by real-time PCR using ethidium bromide monoazide (EMA) has currently been tested. Thus, it is possible to avoid false-positive PCR signals that may be caused by dead cells, e.g., after pasteurization. Moreover, real-time PCR assays for the detection of brewery-relevant wild yeasts (*S. diastolicus*, *S. bayanus*, *Dekkera* spp., *Pichia* spp., *Zygosaccharomyces* spp.) are being developed at the moment. Since for the detection of wild yeasts (*Saccharomyces* and non-*Saccharomyces* wild yeasts), there is no unique cultivation method available up to now. PCR based methods will improve and facilitate wild yeast identification.

Andreas Brandl was born in 1973. From 1993 to 1995, Andreas was a technical graduate as a brewer at the brewery Aldersbach. From 1995 to 2001, Andreas studied brewing and beverage technology at the Technical University Munich-Weihenstephan. Since 2001, Andreas has worked on his doctoral thesis at the Chair for Brewing Technology II at the Center of Life Sciences and Food Science in Weihenstephan. In the framework of his Ph.D. thesis, he is engaged in the EU-Project "Development and demonstration of polymerase chain reaction (PCR) based methods for process control in the brewing industry".

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A New Improved PCR Method for the Detection and Identification of Live Spoilage Organisms

KARIN M. D. PAWLOWSKY, Samantha Walker, and John R. M.

Hammond

Brewing Research International

Spoilage of beer by microorganisms is of great concern to the brewer. Consequently, the need for rapid and reliable microbiological detection methods is ever present. While traditional microbiological methods for the detection of beer-spoilage organisms are extremely accurate and sensitive, they are laborious and slow. Methods based on the polymerase chain reaction (PCR) can be of great help here since they are highly specific and provide results much faster than traditional microbiology techniques. These methods are increasingly being adopted by the food and beverage industries for high throughput analysis and microbiological troubleshooting. One of the drawbacks of PCR methods is the inability to differentiate between live and dead cells. This can be partially overcome by the use of a pre-enrichment step, where the sample is incubated in liquid medium for a short period. While the pre-enrichment step increases the number of live cells, it adds 1–3 days to the time elapsed before detection and has the drawback that the initial cell concentration cannot be calculated. Recently, a novel method for live/dead cell determination has been described. This method has been adapted for brewing samples and shows promise in initial laboratory studies. Ethidium bromide monoazide (phenanthridium, 3-amino-8-azido-5-ethyl-6-phenyl bromide) or EMA is a chemical agent that can traverse dead cell membranes and bind irreversibly to DNA. The bound DNA cannot be amplified by PCR, thus preventing the detection of dead cells. This methodology has been tested using *Saccharomyces cerevisiae* and *Lactobacillus brevis* cells. A range of cell inactivation methods were investigated employing the EMA-PCR technique. For example, cultures of the organisms were pasteurized, EMA was then added and PCR carried out. No PCR product was detected, indicating that EMA did indeed inhibit the amplification of dead cell DNA. The EMA-PCR technique was tested using two different PCR methodologies, standard PCR and real time PCR, with detection by gel electrophoresis and fluorescence, respectively. In both cases, the results

were good. Real-time PCR was the more sensitive of the two methods and allowed discrimination between live and dead cells in mixed cultures. Clearly, this technique should reduce the 'false-positive' signals from dead organisms, often obtained with current methods. The evolution of live/dead assays for the quantitative detection of beer-spoilage organisms by real-time PCR represents one of the most exciting developments in brewing microbiology over the past few years. The EMA-PCR technique could provide a simple, cost-effective method for the rapid detection and identification of viable beer-spoilage microorganisms.

Karin Pawlowsky studied physics in Germany and obtained an M.Sc. degree in molecular biotechnology from Leicester University in the U.K. She then worked in research at the Food Science Department at Leeds University before joining Brewing Research International (BRI) in 1998. At BRI, she has been involved with consumer trials studying beer mouthfeel and drinkability. Later, she moved to the Raw Materials Team, where she worked on lipid-binding proteins in barley and malt. Currently, she is in the Process Team working in the area of molecular biology.

O-59

Detection and Identification of Beer-Spoilage Bacteria Using Real-Time PCR

Cordt Grönwald (1), MATTHIAS KIEHNE (1), and Frédérique Chevalier (2)

(1) BIOTECON Diagnostics GmbH, Germany; (2) Roche Diagnostics GmbH, Germany

For some years, real-time PCR has been used by several breweries on a routine basis to detect beer-spoilage bacteria more rapidly than with conventional, i.e., cultural, methods. The first generation of PCR chemicals allowed the detection of single organisms or, in a later version, a group of bacteria, e.g., all lactic acid bacteria. With the LightCycler foodproof Beer Screening Kit in combination with the dedicated sample preparation kit, Roche offers a new generation of PCR kits now detecting and identifying specifically the group of obligatory beer-spoilage bacteria in one single test. The newest kit comprises primers and probes for the detection of the most relevant beer-spoilage bacteria worldwide (22 species and subspecies). It also allows the identification of the most troublesome organisms in the same test without additional efforts by using the melting curve analysis of Roche Diagnostics' LightCycler instrument. The time to result of the method is about 48 h compared with more than 5 days with classical microbiology. The analytical procedure has been adjusted to the requirements of routine QA laboratories of breweries. Different sample preparation procedures were compared and tested for their applicability in a routine laboratory. The different procedures tested (three) were two manual procedures, one with and the other without DNA purification, and an automated sample preparation using Roche Diagnostics' MagNA Pure LC. The comparison was done by analyzing a broad range of samples from different breweries, e.g., pitching yeast, fermenting beer, finished products, water, CIP solutions, glue, etc. The method also is used to identify bacteria more specifically than is possible with other methods. This identification helps to control and monitor the hygiene status of the complete process from raw materials to auxiliary materials and finished products as well as trace sources of contamination. Different isolates of important species from several breweries and culture collections were tested and the reproducibility of the identification via melting curve was determined. The new tool allows the easy introduction of PCR into a brewery's routine testing. It reduces the time to result and, by identification of the contaminant, it allows the optimization of the hygiene measures during the entire brewing process.

Matthias Kiehne has studied bioprocess engineering at the Technical University of Berlin, Germany. He finished his Ph.D. degree in 1996 while already employed at BioteCon, the predecessor of BIOTECON Diagnostics, a German-based company engaged in molecular diagnostics in the food and beverage industry. Since 1998, he has been responsible for the marketing and sales of the brewing and beverage area of the business and, since 2001, has been head of marketing and sales at BIOTECON Diagnostics.

O-60

Evaluation Study of the Actual Frequency of Different Beer-Spoiling Bacteria with the VIT Analysis

Karin Thelen, Dr. Claudia Beimfohr, and DR. JIRI SNAIDR
vermicon AG

In a recent evaluation study, the trace detection of beer-spoiling bacteria with the rapid detection system VIT-Bier plus *L. brevis* was approved with 500 different brewery samples taken from ongoing production of a large South German brewery. The results that were obtained with VIT-Bier after a short pre-enrichment of 2 days were compared with the results of the conventional analysis after an enrichment time of 7–9 days. The VIT-Bier plus *L. brevis* method was found to be of equal sensitivity for detecting traces of beer-spoiling bacteria, like the applied standard detection method, even though all results were obtained at least 5 days earlier. In addition, the analyzed samples were used to broaden the knowledge regarding the actual frequency of contamination regarding the different beer-spoiling bacteria. This screening analysis revealed that *Lactobacillus brevis* is the most common beer-spoiling bacterium. It was identified in 77% of all samples with positive findings. After this, *Lactobacillus plantarum* (10%) and *Lactobacillus buchneri* (7%) were identified to be the next abundant contaminants. In the frequency of contamination, *Lactobacillus lindneri* and *Pediococcus damnosus* played a minor role. Both were detected in 3% of all analyzed samples with positive findings. The obtained data were compared with already existing data regarding the frequency of contamination in German breweries and a good match of these results was found. The information about the distribution can be a very helpful tool to assess the risks of a potential spoilage with a certain kind of bacterium and will help to conclude in practice what kind of appropriate measure should be taken.

Dr. Jiri Snaidr received his master's of biology degree at the Technical University in Munich, Germany. After studies at the Technical University in Munich, as well as at the Max-Planck Institute in Bremen, Germany, he received his Ph.D. degree in 1997. His work was about the application of molecular biological methods for the detection of hitherto unknown microorganisms. In 1999, he started his studies at the Open University in England and received his degree for senior management in 2000. From 1997 until today, he has been the CEO/president of the vermicon AG in Munich. Dr. Jiri Snaidr founded the vermicon AG in 1997 and focused the company on the development and distribution of test kits for rapid and specific detection of microorganisms. In 2000, the Henkel KGaA took a minor share in the company. In 2001, the first product of a series of subsequent test kits for the detection of microorganisms was launched on the market. In 2003, RWE as well as the energy supplier MVV Energie acquired shares in vermicon. The company is today considered to be a international important supplier of microbiological rapid tests based on leading gene probes technologies.

TECHNICAL SESSION X: Fermentation

Moderator: Dave Ryder

David S. Ryder is vice president—brewing, research and quality assurance at Miller Brewing Company. David began his brewing career in England at Associated British Maltsters. He then joined the South African Breweries Beer Division and was later named director of research & development for that group's brewing and malting concerns at the Delta Corporation Ltd. David was subsequently technical consultant with Artois Breweries in Belgium. Before joining Miller Brewing Company, he was vice president—technical services at J.E. Siebel Sons' Co. Inc., Chicago, and director of education of the Siebel Institute of Technology. David is a member of the Brewing Science Group of the European Brewery Convention (EBC) and currently chairs the subgroup for studying emerging fermentation systems. He is past president of the American Society of Brewing Chemists (ASBC) and past chair of the Program Committee (1988–1992) and the Publications Committee (1992–1994). He has also served on the editorial boards of the Journal of the ASBC and the Journal of the Institute of Brewing. He is past chair of the International Section of the Institute and Guild of Brewing (IGB). He is also a member of the Master Brewers Association of the Americas (MBAA). David has published widely in the brewing literature, which includes the Proceedings of the European Brewery Convention, the EBC Monograph Series, Journal of the ASBC, Brewers Digest, Bebidas, Beverages, and the MBAA Technical Quarterly. In 1982 and again in 1994, he was coauthor of papers that won the MBAA Presidential Award in Brewing.

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New Results with an Immobilized Yeast System: Secondary Fermentation with IMMOPORE

DR.-ING. GERRIT BLÜMELHUBER (1), Univ.-Prof. Dr.-Ing. Roland Meyer-Pittroff (1), and Dr.-Ing. Frank Nitzsche (2)

(1) Technische Universität München-Weihenstephan, Lehrstuhl für Energie und Umweltechnik der Lebensmittelindustrie, Freising, Germany; (2) Easyproof Laborbedarf GmbH, Voerde, Germany

Due to the development of IMMOPORE in 1999, there is an inexpensive carrier material based upon glass for immobilized yeast systems for secondary fermentation. Such immobilized yeast systems have been well-known since the work done by E. Pajunen and J. Kronlöv. While at the beginning, we tried to use multilayer capsules with alpha-acetolactate-decarboxylase to convert 2-acetolactate to avoid a thermal treatment, now we are able to use IMMOPORE as well as a carrier for the yeast and for immobilized enzyme. Therefore, we had to make a small modification on the surface of IMMOPORE. The main principle in the modified plant scheme is based on the known plant layout for immobilized yeast systems for continuous beer maturation. After removal of yeast with a centrifuge, the yeast-free green beer will pass a flash pasteurizer for biological safety (duration of treatment about 30 s). Then it passes a reactor that is filled with alpha-acetolactate-decarboxylase immobilized on IMMOPORE. The major advantage of this carrier material is that the enzymes have a very high activity compared with conventional glass immobilized enzymes. The immobilized alpha-acetolactate-decarboxylase will convert 2-acetolactate into acetoin. For the conversion, there is no thermal decomposition necessary and, therefore, only for biological safety does a short thermal treatment of the green beer have to be done. Tasting tests have shown that this "converted" green beer has the same quality as the original green beer. This is different from the heat treatment process, where a marked change of beer taste is obvious. After enzymatic conversion of 2-acetolactate, the green beer will pass a second fermenter, where the removal of the green beer flavor will take place. In this fermenter, IMMOPORE is placed with immobilized yeast for maturation. The duration of the process is not different from the "old" process layout. It takes about 10 min for conversion of 2-acetolactate to acetoin in the first reactor and about 2–3 h for the maturation process in the second fermenter. A pilot plant has been built, and the resulting beers show a very high quality. This plant layout offers the opportunities first to produce beer at "cold" temperatures within 5–6 days and, second, the use of this novel carrier material offers for the first time a cost-effective industrial-scale secondary.

Gerrit Blümelhuber, né Höhn, received a Dr.-Ing. degree from the Technische Universität München in 2002. After studying brewing and beverage technology at the Technische Universität München-Weihenstephan, in 1996, he began employment at the Chair for Energy and Environmental Technologies in Foodstuff Industry. Since 1998, he is a scientific assistant there.

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Solid State Fermentation (SSF)—Alternative Fermentation Creating Greater Added Value for Grains

MARK LYONS
Alltech Inc.

Many Japanese traditional food and beverage products such as sake, miso, and soy sauce are produced using solid state fermentation (SSF). SSF has also been used for many centuries as a way to utilize waste agricultural materials and to improve the protein content of grains. This paper will review the basics of SSF, including its historical origins in Japan and how these beginnings led to the evolution of SSF globally. Relevant applications in the ethanol industry, such as cellulose conversion, will be discussed. The intricacies of one such solid state process (a fungal species grown on wheat bran under sterile conditions) and the extraction of enzymes from this solid fermentation medium, will be discussed. Solid state enzymes currently manufactured include commercial amylases and proteases, which are now routinely used by the ethanol industry globally. The Alltech SSF plant in Serdan, Mexico, will be used as a case study. This state-of-the-art facility has been in commercial production for 3 years and is the only SSF facility for enzyme production in North America.

Mark Lyons studied at the University of Chicago followed by postgraduate work at Heriot Watt University, Edinburgh. He received his master's degree in brewing and distilling from Heriot Watt in 2000. He is currently the director of Alltech Serdan, a plant extract and enzyme production facility located in Mexico. The solid state component of the plant was

designed and developed under his guidance. He is dedicated to expanding the area of solid state fermentation to incorporate additional by-products and to expand the technology to new applications.

O-63

Primary Beer Fermentation by PVA-Immobilized Brewing Yeast in a Gas-Lift Bioreactor

VIKTOR A. NEDOVIC (1), Dejan Bezbradica (2), Bojana Obradovic (2), Ida Leskosek-Cukalovic (1), and Branko Bugarski (2)

(1) Department of Food Technology and Biochemistry, Faculty of Agriculture, University of Belgrade, Serbia and Montenegro. (2) Faculty of Technology, University of Belgrade, Serbia and Montenegro

Immobilized yeast technology is nowadays a well-established technology for beer maturation and alcohol-free beer production, while its application for primary beer fermentation is still under scrutiny on the laboratory or pilot level. The most commonly used yeast carriers in these processes are DEAE cellulose, porous glass, silicon carbide, and recently, wood chips. These carriers provide a simple immobilization procedure and good mechanical properties but are limited by relatively low cell concentrations and significant cell leakage. Porous matrices present an alternative solution for yeast immobilization providing higher cell concentrations and better cell retention. In the current work, we have studied the application of LentiKats® (polyvinyl alcohol particles produced by a simple gelling technique at a room temperature) for immobilization of brewing yeast and primary beer fermentation in a gas-lift reactor. Viability and growth potential of immobilized yeast were assessed in 6-day cultivation studies in complete growth medium in shake flasks. Activity of immobilized yeast was tested in fermentations of industrial wort in internal-loop gas-lift bioreactors of 1 and 3 L of working volume. Results of growth studies indicated that the lag phase was longer for immobilized cells than for cells in suspension. In addition, the specific growth rate was around 50% lower for immobilized cells. Nevertheless, high cell concentrations were achieved in LentiKat® carriers due to a prolonged exponential phase of immobilized cells. Fermentation studies in gas-lift bioreactors demonstrated high fermentation activity of immobilized cells. Apparent attenuations in the range of 80 to 86% were achieved for less than 24 h with solid loading of around 10% mass. The quality of the obtained beer was comparable to the quality of beer produced by suspended cells. Concentration of cells in carrier was 1.4×10^9 cells/mL, while the final concentration in medium was 2.1×10^7 cells/mL. Results of this research indicated LentiKats® was a suitable carrier for brewing yeast cells, especially when applied in gas-lift reactors. This carrier, due to its lenticular shape, provided good mass transfer properties and an easier and more efficient separation procedure from the fermentation broth as compared with microbead carriers. In addition, LentiKats® provides excellent mechanical properties, which extends its potential applications to long-term and large-scale continuous beer fermentation processes.

Viktor A. Nedovic received B.Sc., M.Sc., and Ph.D. degrees in food technology, biochemical engineering, and biotechnology, respectively, from Belgrade University in Belgrade, Serbia. He is employed at Belgrade University, Department of Food Technology and Biochemistry, as assistant professor for the subjects technology of beer and malt production and biochemical engineering. He has conducted several research projects in the fields of beer fermentation, immobilization and bioencapsulation of cells, and bioactive molecules. He is a member of Management Committee of COST 840 Action (Bioencapsulation Innovations and Technologies). He is a member Bioencapsulation Research Group (BRG) and founder and secretary general of the Biochemical Engineering Society. He has served as coeditor of two major books (publisher: Kluwer Academic Publishers) covering the fundamentals and applications of immobilized cell technologies: Fundamentals of Cell Immobilisation Biotechnology (published in Dec. 2003) and Applications of Cell Immobilisation Biotechnology (in press). Recently, he has served as guest coeditor of the special issue of the journal Chemical Industry, which was devoted to the 11th BRG Conference "State of Art of Bio&Encapsulation Science and Technology", held in Strasbourg, 2003. He has served as reviewer of scientific papers for the journals Biotechnology and Bioengineering, Journal of Agricultural and Food Chemistry, and Food Microbiology.

O-64

Detection of Beer-Spoilage Microorganisms Using the Loop-Mediated Isothermal Amplification (LAMP) Method

YOUICHI TSUCHIYA (1), Masahiro Ogawa (1), Yasukazu Nakakita (1), Yasunobu Nara (1), Hirotaka Kaneda (1), Masachika Takashio (1), Harumi Minekawa (2), and Takahiro Soejima (2)
(1) Sapporo Breweries Ltd.; (2) Eiken Chemical Co., Ltd.

Draft beer is an area of market growth in many parts of the world, which has made the biological monitoring of beer-spoilage microorganisms even more important. Loop-mediated isothermal amplification (LAMP) developed by Eiken Chemical Co., Ltd. (<http://loopamp.eiken.co.jp/e/index.htm>) is a nucleic acid amplification method that reacts under isothermal conditions and produces large amounts of DNA. The LAMP method requires a set of four specifically designed primers and a DNA polymerase with strand displacement activity. The amplification products are stem-loop DNA structures with several inverted repeats of the target and cauliflowerlike structures with multiple loops. Thereby, after the LAMP reaction, white precipitates identified as magnesium pyrophosphate are observed in the reaction mixture; thus, the presence of these precipitates confirms that DNA was amplified. We designed the primers specific to 11 species of yeasts and bacteria defined as spoilers for our products, optimized conditions for LAMP, and developed the rapid detection and identification kit containing all these primers in one mixture. When LAMP was carried out using this kit at 63°C for an hour and a half, all beer spoilers could be specifically detected by generation of the white precipitates in the reaction mixture. *Lactobacillus brevis* and *Pediococcus damnosus* are known as representative beer spoilers and include beer-spoilage as well as nonspoilage strains. The primers specific to the beer-spoilage strains were designed based on the sequence of the *gyrB* gene, including sequence alteration between beer-spoilage and nonspoilage strains (1). *Saccharomyces* species include both bottom fermenting yeasts and beer-spoilage wild yeasts. Optional primer sets based on the sequence of the *MEL* gene were also prepared to distinguish them. This kit is simple and easy to perform, requiring only a regular water bath or heat block for reaction. Given the simplicity and cost-effectiveness of the reagents and equipment involved, LAMP has an advantage over PCR requiring the expensive real-time instrument or a regular thermal cycler with additional detection system such as electrophoresis or ELISA. The LAMP should contribute greatly to microbial quality assurance in breweries. 1. Nakakita, Y., Maeba, H., and Takashio, M. Grouping of *Lactobacillus brevis* strains using the *gyrB* gene. *J. Am. Soc. Brew. Chem.* 61:157-160, 2003.

Youichi Tsuchiya was born in 1963. Youichi graduated from Kyoto University, Japan (Department of Food Science and Technology, Faculty of Agriculture) with a bachelor's degree, 1987; a master's degree, 1989; and a Ph.D. degree, 1999. Youichi's Ph.D. thesis was 'Application of genetic analyses to brewing'. Youichi joined Sapporo Breweries Ltd. in 1989 and was with the Quality Assurance Department, Brewing Research Laboratories until 1996; the Microbiology Department, Brewing Research Laboratories until 2002; and the Frontier Laboratories of Value Creation as lead microbiologist until the present.

O-65

Genetic Characterization of Hop-Sensitive Variants Obtained from Beer-Spoilage Lactic Acid Bacteria

KOJI SUZUKI, Kazutaka Ozaki, and Hiroshi Yamashita
Asahi Breweries, Ltd.

A limited number of species belonging to lactic acid bacteria represent the majority of beer-spoilage bacteria. Most species of lactic acid bacteria fail to grow in beer because the hop compounds, added to confer bitter flavor to beer, is the major neutralizing agent. Interestingly, some strains belonging to one species have a strong hop-resistance ability and are capable of vigorously growing in beer, whereas some others, belonging to the same species, have no beer-spoilage ability. Nonetheless, the hop-resistance ability has been reported to be a stable character, and the origin of beer-spoilage lactic acid bacteria is one of the mysteries for brewing microbiologists. In this study, we attempted to obtain a hop-sensitive variant from beer-spoilage *Lactobacillus brevis* ABBC45. As a result, it was shown that the incubation temperature, higher than optimal, caused the permanent loss of the beer-spoilage ability of this strain. Genetic characterization of the non-beer-spoilage variant revealed that two genetic regions, containing *horA* and ORF5, respectively, were lost concomitantly with the loss of beer-spoilage ability. It was found that *horA* and ORF5 were carried independently by two plasmids, designated as pRH45 and pRH45 \ddagger U, and the presence or absence of these two genetic markers were

highly correlated with the beer-spoilage ability of various species of lactic acid bacteria. Strikingly, all the beer-spoilage lactic acid bacteria in our culture collection were found to possess at least one of the two genetic markers, indicating that the beer-spoilage ability can be determined by these *trans*-species genetic markers. The sequencing analysis of *horA* and ORF5 homologs, identified in various beer-spoilage species, revealed approximately 99% nucleotide sequence identity with those of *L. brevis* ABBC45. These results indicate that *horA* and ORF5 have not evolved with the speciation processes. Indeed, the loss of *horA* and ORF5 occurred with the loss of hop-resistance ability of seven beer-spoilage strains other than *L. brevis* ABBC45, suggesting that these markers are not innate genetic regions. Taken collectively, these *trans*-species genetic markers are most likely to be acquired by horizontal transfer and confer the beer-spoilage ability on originally innocuous lactic acid bacteria. This insight gives a theoretical foundation for using *trans*-species genetic markers for differentiating the beer-spoilage ability of lactic acid bacteria.

Koji Suzuki received an M.S. degree in agricultural chemistry from Tokyo University in Tokyo, Japan. He began employment with Asahi Breweries, Ltd. in April 1992 as a microbiologist. Since April 2003, he has functioned as chief researcher in the Analytical Technology Development Section of the Analytical Technology Laboratory.

O-66

Hygiene Monitoring in the Food Industry—A New Approach for Control of the Microbiological Situation

DR. FRANK NITZSCHE
Koenig Brauerei GmbH

Stable processes for the production of food are necessary for a safe production. Upcoming regulations require known processes without the risk to deliver unsafe products. Beside the risk of a chemical contamination that might be detected, very fast microbiological contaminations might cause a major damage to the health of the consumer. But, as a major disadvantage, microbiological analysis methods last at least several days. Due to the problem of growth prior to the detection of the microorganisms, the analysis result will be available too late. Different strategies have to be set up in a food-producing company to keep the number of microorganisms as low as possible. Beside the reduction of the growth of biofilms in pipes, the risk of secondary contamination has to be decreased. For the first question, the internal stabilization of the supplied water with the help of chlorine dioxide might be a helpful choice. The reduction of the risk of the secondary contamination within the filling lines might be lowered due to better education of the operating personal. Increasing the knowledge of personal hygiene through education of the workers together with simplified pictures and demonstration of growing germs enable them to be in more hygienic working conditions. Second, the optimization of the technical equipment with cheap but successful mechanical improved cleaning processes (e.g., short washing steps within the filling plant will help to reduce the availability of organic material as a nutrition and growing source) will reduce the risk of a secondary contamination. In combination with hot-water rinsing steps, two major basic conditions for growing of germs will be removed. The next step in optimization of the "stabilization" process, the work with chlorine dioxide, will decrease cleaning costs and increase cleaning results. Chlorine dioxide is a gas that is soluble in water. It is allowed to be used as a water stabilization agent, but the use as a disinfectant without any negative influence on flavor or other product properties is possible. Chlorine dioxide produces no organic chlorine compounds, such as chlorophenols or halogenated hydrocarbons. Introducing of new methods in the laboratory for detecting live germs without pre-enrichment will be the next step on the way to increased product quality at cheaper costs with increased product safety. This fast microbiology allows the production plant to optimize the cleaning processes in real time. This will allow the opportunity to save cleaning costs and to reduce the environmental impact (water and cleaning agent consumption).

Dr. Frank Nitzsche (born in 1960) studied to become a brewer and malster at Veltins Brauerei, Germany, between 1981 and 1983. He received a master's degree in brewing science from TU Muenchen-Weihenstephan in 1988. His Ph.D. work was done with Prof. Dr. Narziss until 1991. Since then he has been working for Koenig Brauerei as head of the R&D department at Koenig Brauerei until 1994, as head of QA until 1997, and nowadays, he is responsible for production and QA. In 1999, he

founded Easyproof Laborbedarf GmbH, which works mainly on fast microbiological detection methods.

TECHNICAL SESSION XI: Packaging

Moderator: Christopher Nunes

Christopher Nunes is a graduate from The University of Toronto in biochemistry and has a diploma in brewing technology from the Siebel Institute of Technology. He has worked for Molson Breweries for the last 20 years in packaging, maintenance, continuous improvement, and reliability. Chris is currently the director of continuous improvement at the Molson Toronto Brewery.

O-67

Withdrawn

O-68

Development of a Hybrid Canning Line with High Productivity in a New Kyushu-Kumamoto Plant

KOICHI HOTTA

Suntory Ltd., Kyushu-Kumamoto Plant

Japanese beer companies are recently turning into total beverage companies that also produce soft drinks. In order to meet this demand, our Kyushu-Kumamoto Plant started production in April 2003 as a total hybrid beverage factory including beer, soft drinks, and in future, low alcoholic beverages. Because this is our only factory on the Kyushu Island of Japan, it is required to produce all kinds of products consumed in the area and to deliver them quickly to the customer. Therefore, high flexibility of production is strongly required for the factory. Packaging section consists of three lines, that is, PET bottling, canning, and kegging lines. They are basically designed to handle all products except for aseptic ones. The canning line is a hybrid line of beer and soft drinks, such as coffee, oolong tea, etc. Therefore, it should produce various kinds of products with relatively small volume. It is essential to achieve quick product change while keeping good product quality. First of all, to achieve quick changeover, two fillers have been introduced into the line to run alternatively. One filler is for beer and the other one is for soft drinks. This contributed to the reduction of shutdown time for CIP, especially long ones to prevent flavor contamination among different products. Secondly, a block changeover system was adopted. Hybrid line has a problem of trip time difference, depending on the products. Beer cans pass through the line in 1.5 h, while it takes 3 h for coffee cans. As a solution, the whole line was divided into eight blocks and the product in each block can be changed independently. To keep good product quality, counter-pressure of the filler bowl during beer introduction and CO₂ gassing before seaming are precisely controlled automatically. As a result, in spite of frequent product changes, CO₂ volume and O₂ pickup are controlled within a small deviation. Next, to deal with complexity of materials, a pallet identification system with QR code was adopted. Each pallet of material has a unique code on it. Before getting into use, they are compared with the production plan and misuse of different materials has been prevented. And in order to raise the online detection ability, an empty can inspector with high flexibility was installed with great success. Furthermore, about 4,000 signals and data, such as temperatures, pressures, and motion of valves are collected and they are easily accessed from every PC in the office. With these measures, Kumamoto canning line is handling more than 10 kinds of cans and producing twice as many kinds of products a week as the other factories. At the same time, high level quality assurance has been achieved.

Koichi Hotta received a master's degree in mechanical engineering from Kyoto University in Japan. He began employment with Suntory in April 2000 as a mechanical engineer in the packaging section of the Tonogawa Brewery. In August 2001, he became a member of the new Kyushu-Kumamoto Plant Installation Project. In this project, he was in charge of designing the entire packaging section. Since it started production, he has been working as a technical staff member of the plant.

O-69

Plastic Beer Bottles: Where Are We Today?

NINA GOODRICH

Amcor

Plastic beer bottles have been available commercially for the last 5 years but have only begun to gain momentum. North America has seen a few introductions, but Eastern and Western Europe have been more active. This talk will focus on what has changed? It will provide an overview of the technology improvements required to fuel new market development and sources of these technology changes. Specifically, it will cover the following. • Plastic's impact on the environment. Plastic offers some sustainable lifecycle options that are different than glass. • Product protection. Barrier has always been a challenge when we consider beer in plastic. Measurement of barrier, barrier options for oxygen, light, and carbon dioxide will all be covered. • Shapes and sizes: What shapes are possible? Plastic offers new decoration options and differentiation options. • Cost. Security of supply and resin stability will be addressed. • Process compatibility. To pasteurize or not to pasteurize? What are the plastic bottle implications? What does it mean for filling lines? It will also include a review of current launches globally.

Nina Goodrich is the director, value creation and business innovation for Amcor PET Packaging, in the Diversified Products Division. In the past, Nina has led Amcor PET's Centre for Technology. This group developed one of the first commercial barrier plastic beer bottles in North America. It pioneered the use of a monolayer active scavenging bottle. In this technical role, Nina was very active in the development of recycling systems for sustainable bottle-to-bottle recycling. Prior to joining Amcor, Nina was the director of operations for the Guelph Food Technology Centre. Nina has a degree in molecular biology from Wellesley College and has done graduate work in management. She is a frequent speaker at food and packaging industry events and conferences.

O-70

Shortening the Changeover Time of a Can Line into Less than 10 Minutes

TAKAHIRO YONEDA and Go Hasegawa

Asahi Breweries, Ltd.

In the Japanese market, bottles, cans, and kegs are used for beer containers. Among them, 350- and 500-mL cans are the most popular in the market. Besides a case of 24 cans, they are sold as Loose with individual 24 cans, and Multi Pack with bundled 6 cans. Therefore, the four most popular packaging forms are 350-mL Loose, 350-mL Multi Pack, 500-mL Loose, and 500-mL Multi Pack. Asahi Breweries, since 1992, has worked on fresh management activities, and the main brand, Super Dry, has increased its market share. In order to improve freshness of the beer, each brewery is required to produce the suitable beer volume in the number of containers demanded in the market every day. However, the changeover time between the 350- and 500-mL can forms in the line used to be 300 min, and the time to switch between Multi Pack and Loose used to be 60 min. It was inefficient to produce the above four kinds every day. Therefore, we pooled our efforts to achieve the goal of shortening the time to less than 10 min in order to provide more fresh beer to the market. As for three basic policies of our efforts, first, those works that only manpower is able to do should be kept as is. Examples are to check leftover cans of the previously processed kind, the quality of contents and packaging, and the operation status of each inspector. Second, automation with IT and machinery should be taken with the consideration of profit. Third, the established works should be reconsidered in terms of time reduction. Regarding the actual measures, we introduced, even developed, the automatic changeover systems with the pneumatic or electromotive powers, instead of manpower. This made the time shorter as well as reduced troubles resulting from the changeover. Another is to divide one line into several areas. Instead of finishing one consecutive works in a line for one kind, when we finish works of one area for one kind, we change into the different kind to do the same works of that area. Also, we introduced automation in changeover and production starts. Instead of human judgment, we set up a system that judges the conditions for automated changeover and start by networking necessary equipment. Additionally, we introduced swivel joints in the product in-feed pipe toward the filling machine, since we cannot expose the nonsterilized part that directly touches beer to the air while changing filling machines. Finally, these alterations raised operators' consciousness positively on changeover and made them perform many efforts for improvement called *Kaizen*. Therefore, we achieved the goal of shortening the changeover time to less than 10 min.

Takahiro Yoneda received a B.S. degree in chemical engineering from Kyoto University in 1990 and started his job in the central research center of Asahi Breweries, Ltd. During the first 4 years, he researched and developed packaging materials such as metal cans and PET bottles with strength, among other assignments. Also, he developed the direct distilling system in draft kegs. While working at five different locations, he also engaged in production, being in charge of startup and responses in packaging lines. In 2000, he studied packaging at Michigan State University as an exchange-visiting scholar for a year.

O-71

Development and Introduction of High-Performance Full-Bottle Inspector

HIROHIKO INOUE and Hirohisa Suzuki
Asahi Breweries, Ltd.

The requirements of bottle packaging are correct fill level, secure crowning, no foreign substance in the beer, cleanliness of bottles, no cracks or scuff on bottles, and others. The recovery rate of beer bottles in Japan is high, and most of the bottled beer products are packed in the recovered bottles. About 5% of recovered bottles is defective. The consumers have high expectations toward product quality, and we manage the production processes so as not to produce any defective products that can induce customer complaints. Asahi Breweries had performed empty bottle inspections before and after rinsing by machines and visual bottle inspection by manpower. In building the new Kanagawa Brewery in 2002, the visual bottle inspection was abolished and the full-bottle inspector was introduced. In the same year, we, together with Matsushita Electric Works, developed a type of the full-bottle inspector that is compatible with our other breweries. In November 2003, two of our breweries introduced it. During development, we reconsidered the requirements in the former bottle inspections, which are newly included in the design of the new full-bottle inspector. The defects in recovered bottles and insufficiency in rinsing are discovered and managed at the rinser and the empty-bottle inspector. Therefore, the full-bottle inspector can mainly focus on checking the defects that can be generated in filtration and crowning. The focus made more effective and precise inspection possible. Also, we considered the stability in conveying bottles, maintenance, information processing of data of inspection, and pictures to make the inspector usable. Regarding outlines of the inspector, the maximum inspection speed is 720 bpm, and the conveyor is a single and linear line. The inspection items are foreign substances, leakage, cracks in the bottle lip and bottom, fill level, and crowns. The inspector can analyze data of the numbers of defects and can save the pictures of defects. The competence of the inspector is proved at the two breweries, since they achieved the goals identified in the beginning. The inspector raised productivity and reinforced the quality management system. Furthermore, it is planned to be introduced to our other breweries.

Hirohiko Inoue received a B.S. degree in mechanical engineering from Kagoshima University in Japan. He began his employment with Asahi Breweries, Ltd. in 1990. For 9 years, he was engaged in engineering of the production facility (on brewing and packaging) in the breweries. Since 1999, he has been with the Technology Department of Asahi Production Headquarters. He has worked on raising productivity of packaging lines.

O-72

Impact the Bottom Line: A Business Case for Reliability-Driven Maintenance

Christopher Nunes (1) and PAUL LANTHIER (2)
(1) Molson Canada, Toronto, ON, Canada; (2) Ivara Corporation,
Burlington, ON, Canada

Many external factors are forcing brewing companies to focus on cost efficiencies to gain a competitive advantage. Maintenance organizations are now being charged with the responsibility to contribute to the bottom line. The condition, availability, and reliability of plant assets affect a brewing company's ability to meet fluctuations in market demands, maintain quality and safety levels, and minimize product losses. Improving asset reliability impacts the bottom line. This paper discusses how to develop a business case to justify reliability-driven maintenance. The resulting business case assesses the current state of maintenance and clearly identifies the financial benefits/costs and the roadmap to achieving those benefits. • Gain a clear understanding of maintenance performance as it compares to world class standards and the benefits that can be

realized. Look at the way maintenance is conducted in an organization and the degree to which specific requirements support an effective maintenance process. • Learn how to identify financial benefits associated with improving reliability, such as maintenance costs savings, maintenance-related downtime reduction, and waste reduction. Define metrics for current and future state and provide benefit/cost/effort projections. • Develop a roadmap to achieve a reliability-driven approach to maintenance. – Focus on maintenance as a business process. The business process defines what must be done consistently to optimize reliability. – Ensure that reliability practices are established to identify the right work to do on the right equipment at the right time. – Support the data-intensive maintenance business process with technology. – Manage the change from reactive to proactive maintenance to ensure results are sustainable. This paper describes how to realize significant value in adopting a reliability-driven approach to maintenance. By embracing the business process, training, and empowering employees and giving them the right supporting practices and tools, the maintenance organization will work more effectively and, in turn, impact the bottom line.

Paul Lanthier is a graduate from Queen's University in electrical engineering and a certified professional engineer in the provinces of Ontario and Québec. Paul is also a certified RCM2 (reliability centered maintenance) practitioner. Over the past 23 years, he has worked in the reliability engineering, process control and process optimization fields, the material and structural testing field, and the quality inspection field. Paul is currently a member of Ivara Corporation's Reliability Solutions group.

TECHNICAL SESSION XII: Beer/Brewing

Moderator: George Reisch

George F. Reisch is a corporate brewing staff brewmaster for Anheuser-Busch in St. Louis. He is a fifth generation brewmaster. His family owned and operated the Reisch Brewing Co. of Springfield, IL, from 1849 until it ceased operations in 1966. George graduated in 1979 with a B.S. degree from the University of Wisconsin. He was hired by Anheuser-Busch upon graduation and, in 2004, is celebrating 25 years of service to Anheuser-Busch. George is an active member of both the MBAA and the ASBC. He is a past president of MBAA District Southern California and is currently serving on the Education and Technical Committees for the national MBAA office. In addition, he is a member of the Board of Advisors for the North American Brewers Association (NABA). George's current duties include overseeing Budweiser, Bud Light, Busch, and Busch Light production at the Labatt breweries in Canada. George, his wife Kathy, and his four children live west of St. Louis in Wildwood, MO.

O-73

Two Different Brewing Processes Disclosed from Two Ancient Egyptian Mural Paintings

HIDETO ISHIDA
Kirin Brewery Co., Ltd.

Our trial of reproducing two kinds of ancient Egyptian beer was successful. Two different mural paintings were referred to in order to know the beer-brewing scene. One was the painting in Niankhkhnum and Khnumhotep's tomb from the Old Kingdom era (2650–2180 BC, Saqqara) and the other was the painting in Kenamun's tomb from the New Kingdom era (1570–1070 BC, Thebes). Each tool and scene described in these two mural paintings was significant in that they follow the Common Pathway of world alcoholic beverages in detail. The same tools and replicas, such as pots and anforas, were imported from Egypt to conduct the test brew under the same conditions of those ages, which depended on the nature of the clay and the shapes of the pots and anforas. The brewing process in the Old Kingdom era was similar to the modern beer-brewing process in that fermentation was continued to mashing by a single mash decoction process. The brewing process in the New Kingdom era was not similar, because converting starch and fermentation were carried out simultaneously in the same pot. There were significant differences in the purification of yeast between those two eras. People in the Old Kingdom era were baking sour bread to defeat lactic bacteria and were utilizing the remainder of the lactic acid to select suitable yeast from its effect of avoiding bacterial contamination. People in the New Kingdom era found a purification method of yeast by kneading sourdough with alcohol without baking it. Especially, unique fermentation in the solid body was taken in this process. As main yeast, *Saccharomyces cerevisiae* was chosen in both

methods. Date played an important role in yeast selection and in keeping them pure in each case. New Kingdom beer contained malt, full-steamed bread, and unbaked dough. Samuel's SEM observations into residual starch granules of an ancient Egyptian pottery vessel agreed with our process. Since alcohol concentrations of both final beers were over 8% (v/v), it might be possible to transport and stock them at some degree. Niankhhnum's beer tasted like sophisticated white wine with a slightly malty after-note, which was caused by similar concentrations of lactic acid. Although Kenamun's beer was like an alcoholic yogurt drink with a very sour taste and was similar to the Egyptian drink Bouza, it had mysterious charms to make people want to drink it again.

Hideto Ishida graduated in the biochemistry field from Kyushu University in Fukuoka, Japan. He began employment with Kirin Brewery in April 1969 as a chemist in the analytical laboratory of the Yokohama Branch. He has engaged in brewing, malting, QC circle activity, environmental management, corporate technical planning, bottle making, operations research, technical consulting in the China plant, and public relations management in the Kobe plant.

O-74

Miller Valley Brewery as a Development Tool in the 21st Century
JEANNE L. MARAIS, David S. Ryder, Susanne S. Terharn, and Gerald Czernicki
Miller Brewing Company

Miller Valley Brewery (MVB), Miller's state-of-the-art pilot brewery and packaging facility within its Milwaukee Technical Center, started its operations in 2000. The brewing facility has a capacity of 12 hL as wort per batch, complemented by a smaller 40-L brewing system. The MVB is the first of its kind in the U.S. and has enabled Miller to greatly improve its capabilities in new product development. Consumer needs and preferences are becoming more diverse, thus making new product development an area of critical performance. Recent years have shown that survival in the U.S. market requires not only new products but also the diligent evaluation of processes and ingredients to sustain customer satisfaction. It has also shown that not only the product itself, but also the timing, are critical factors. The MVB was designed and built to enable the fastest possible response to market trends and to support critical marketing initiatives. Its cutting-edge technology is used to enhance and accelerate the testing and development of new and existing products and processes without disrupting normal brewery production. The result is a critical savings in time and cost, as well as enhancement in production efficiency. Before 2000, Miller's commercial breweries averaged about 110 brewing trials per year. Now, more than 200 trials are done at the MVB annually, using both novel and traditional technologies to craft products to delight the taste buds of future consumers. In this report, the following topics will be discussed: 1) structure and function of pilot brewing within Miller Brewing Company, 2) MVB design and equipment, 3) MVB successes and achievements, and 4) the outlook for the future of pilot brewing at Miller.

Jeanne Marais received a B.S. degree in chemical engineering from the University of Cape Town in South Africa. She began employment with South African Breweries in 1997 as a technical trainee and, later, was a process engineer at the Ohllson's Cape Brewery in Cape Town, South Africa. She joined Miller Brewing Company in 1999 as a staff brewer in the Corporate Brewing Department at Miller's Headquarters in Milwaukee, WI. Since August 2002, she has functioned as pilot brewing manager in product and process innovation, reporting to Susanne Terharn.

O-75

Design, Planning, and First Practical Experience—The New Grolsch Brewhouse in Enschede, The Netherlands
Guy Evers (1) and THOMAS BUEHLER (2)
(1) Grolsch Innovation and Technical Services Department, Enschede, The Netherlands; (2) The Huppmann Group, Kitzingen, Germany

The paper will present the design and first experience with the new brewhouse at the new Grolsch brewery in Enschede, The Netherlands. Six years ago, Royal Grolsch Brewery decided to invest in a new brewery site. Reasons for the decision to invest into a completely new site were the projected savings in labor and energy as well as environmental and increased safety aspects on one hand. On the other, it was the growth

potential of the brand. The targets will be presented and discussed. In process execution, one of the challenges for Huppmann was to meet the narrow time line. Due to the complexity of the installation, the design phase involved a complete 3D planning of the brewhouse. Despite a fast project execution, brewers and engineers paid much attention to the engineering and technological details. Already in this stage and especially during installation, the investment in 3D planning paid back. The new Huppmann brewhouse, with a total capacity of 4,000,000 hL of wort per year (producing wort in three shifts 5 days a week) is a two stream setup that accomplishes the aforementioned criteria of efficiency and quality. The setup of the plant and the reasons behind the layout will be presented in this paper. During commissioning, which took place at the beginning of 2004, the brewers could gain first experience with the new brewhouse. Important aspects are the adaptation of the beer style to the new brewhouse as well as the performance parameters. The paper will present these results.

Dr. Thomas Buehler started brewing with an apprenticeship as a brewer and maltster. In 1991, he graduated with a Diplom-Ingenieur in brewing from the Technical University of Munich-Weihenstephan. In 1991, Thomas was employed as a scientist at BRI, Nutfield, U.K. In 1995, he started as manager training and technology at APV, Dortmund, Germany. Thomas graduated with a Ph.D. degree in chemical engineering from Loughborough University, U.K., in 1997. From 1996 until 2003, he was managing editor for Brauwelt at Fachverlag Hans Carl, Nuremberg, Germany. Currently, Thomas is director marketing and R&D at The Huppmann Group, Kitzingen, Germany.

O-76

Wort Aeration—A Critical Approach
CHRISTOPH TENGE and Eberhard Geiger
Technische Universität München, Center of Life Sciences Weihenstephan, Chair for Brewing Technology II

A current topic in brewing research is the area of flavor and colloidal stability of beer. Oxygen uptake is considered to be the single largest negative factor affecting stability. Therefore, modern brewing technology endeavors to prevent oxygen uptake from all steps in production, from mashing to bottling. However, in one step of the process, oxygen is desired in large amounts, that is, during wort aeration. The air is introduced into the wort in order to supply the yeast with oxygen. At the beginning of primary fermentation, the yeast requires oxygen for aerobic metabolism and growth and synthesis of membrane lipids. It is more precise to discuss yeast aeration rather than wort aeration because the wort does not require oxygen. Despite preventative measures, such as the introduction of air into the wort at a low temperature and in the presence of yeast, a loss in reductones is detectable. This oxidative deterioration was measured with an electrochemical ITT test and will be presented. As stated above, supplying the yeast with oxygen is essential; however, wort aeration is not the only option. Modern yeast cultivation, such as propagation technology, produces yeast in excellent physiological condition while providing them with an adequate oxygen supply. Will yeast of this quality ferment unaerated wort? In order to answer this question, it was necessary to conduct brewing and fermentation experiments on a laboratory and pilot scale. Technological analyses were carried out, such as degree of attenuation, the formation of metabolic by-products, etc. A sensory evaluation of each of the beers was performed and the flavor stability was tested. These results provide a basis for evaluating the possibility of working with nonaerated worts. The experiments show that by using yeast in good condition, it is possible to ferment nonaerated wort. The degree of attenuation was comparable to beer production using aerated worts. High-quality beers are able to be produced using this method. Additionally, by preserving the antioxidative capacity of the wort, the flavor stability of the beer is enhanced. Results obtained in the laboratory were confirmed using a pilot plant. These initial findings show that it is possible to maintain a high wort quality through shifting aeration from wort aeration to yeast aeration during the propagation process.

Christoph Tenge was born in 1972. From 1991 to 1998, Christoph studied brewing and beverage technology at the Technische Universität München-Weihenstephan, Germany, and earned the degree of Diplom-Ingenieur with a doctoral dissertation of "Developing a technology to produce alternative beverages out of grain extracts with specifically isolated fermentation-organisms" (1999–2002). Christoph has been employed as a

freelancer for molecular biology studies, Dr. Vogeser, D-Tect GmbH in Freising, Germany (1998–1999); research assistant at the Chair for Brewing Technology II, Technische Universität München-Weihenstephan, Germany (1999–2001); and assistant professor at the Chair for Brewing Technology II, Technische Universität München-Weihenstephan, Germany (2001–present).

O-77

Observations on a Lauter Tun with a New Design

PROF. DR.-ING. HEINZ MIEDANER (1), Matthias Weinzierl (2), and Klaus Wasmuht (2)

(1) Staatliche Brautechnische Prüf- und Versuchsanstalt, Freising, Weihenstephan, Germany; (2) Anton Steinecker Maschinenfabrik GmbH, Freising, Germany

Lautering is one of the major steps in beer production. Here, the efficiency of the brewhouse is determined. But lautering also affects the wort quality. The requirements on modern lautering systems rise continuously. Whereas quality and quantity of the extracted wort used to be the main topic, today's recurrent demand is to increase profitability and flexibility as well. Currently, lauter tuns for 10 brews/day are used in many breweries all over the globe. With a little more technical expenditure, 12 brews/day are already state of the art. To take all these points into account, a completely new lauter tun was conceived that surpassed the expected improvements in practice by far. The new lauter tun bears the name of Pegasus, going back to Greek mythology. Pegasus is a winged horse that emerged from Medusa after having been beheaded by Perseus. The name is to illustrate the speed and the quality of the new lautering method. The design of the new lautering system has the following advantages: Even mash storage from center, arrangement and dimensioning of the source areas enable uniform flow conditions, the extract intake is faster, the soluble extract is low, the yield is increased, the weak wort concentration is low, the spent grains are drier, 12 brews/day are achieved with higher specific loads of 14 brews/day are possible, and the total of solids and the turbidity are unobjectionable. This paper illustrates the principle of the system and the results of recent acceptance tests.

Prof. Dr.-Ing. Heinz Miedaner was born in 1941 in Munich, where he spent his childhood and youth. From 1960 to 1965, he studied at the Technische Universität München-Weihenstephan "Brewing Technologie" and graduated as Diplom-Ingenieur. In 1965, he started working as the assistant of Prof. Dr. Ludwig Narziss at the Chair of Brewing Technology and graduated as Dr.-Ing. The thesis of his doctoral was "Influence of barley variety and malting conditions on the formation of higher alcohols and esters during fermentation and storage". From 1969 to 1971, he started working as quality control manager at the Erste Kulmbacher Aktienbrauerei, Kulmbach. From 1971 to 1995, he started working as chief engineer of Prof. Dr. Ludwig Narziss, where he could focus on different working fields, such as practical training of students, lectures in international brewing methods and technological quality control, flavor research (GC and GC/MS), and consultancy of breweries and malting plants. In 1980, he wrote his doctoral with the topic of "Some aspects of fermentation and maturation". In 1990, he was nominated as professor. Since 1995, he is working as director of the Staatliche Brautechnische Prüf- und Versuchsanstalt, Freising, Weihenstephan.

O-78

Today's Small Brewer: An Industry Partner

DANIEL BRADFORD

Brewers' Association of America

Small brewers are the leading edge of a dramatic shift in the marketplace. They accomplished this through a pair of strategies. As the most dynamic part of the industry, small breweries can be found on the leading edge in many areas. Although securely entrenched in the industry, this segment is threatened by the evolving industry political and economic climate. During the past year, the small brewing industry was the fastest growing category of the beer industry. With numbers reported ranging from 3 to 5% market share and up, this category is clearly the star. As trading up to higher-priced brands turned from a trend to an industry pattern, small brewers provided both core brands as the bulk of the business and product innovation that continued to inspire and vitalize the segment. Although there are exceptions to the rule, these brewers continued to introduce a steady stream of diverse, innovative, and exciting brands. During a period when the big news has been spirits branded malternatives and low carbs,

small breweries persisted in stretching the envelope. Beyond style innovations, small breweries are innovators in creating environmentally friendly breweries and using alternative energy sources. Within the brewhouse, small breweries are also known for their innovations in technology, packaging, and marketing. Furthermore, the grass roots passion surrounding small breweries has revived the traditional loyalty for local breweries and inspired a whole new segment of beer "aficionados". Although the most aggressive, innovative, and expanding segment, small breweries face unique challenges. As they expand their market and their consumer base, small brewers face an increasingly restricted marketplace. The globalization of the beer industry has elevated the competition. The structure of the distribution and retail channels makes it more difficult for small brewers to successfully reach consumers. In conclusion, domestic small brewers are providing the industry with positive impacts in all areas. However, consolidation in the three-tier system is making it increasingly difficult for small breweries to access the market and succeed.

Daniel Bradford assumed his current position, president of the Brewers' Association of America, in September 1999. He is responsible for directing the activities of the small brewing industries' trade association, including political, promotion, and advocacy. Prior to holding this position, Daniel published All About Beer Magazine for 10 years. One of the earliest consumer beer magazines, All About Beer Magazine now ranks as the leading magazine on beer. Before purchasing All About Beer Magazine, Daniel was the marketing director for the Association of Brewers and the director of the Great American Beer Festival. He has published articles on beer in most beverage trade publications in the United States, Great Britain, and Germany. He is also a partner in Top of the Hill Brewery, Chapel Hill, NC. His wife edits All About Beer Magazine and contributes a weekly column on beer to the Raleigh News & Observer.

TECHNICAL SESSION XIII: Yeast

Moderator: Greg Casey

Greg Casey, born and raised in Toronto, Canada, graduated from the University of Guelph in 1979 with a B.Sc. degree in applied microbiology, continuing on to obtain a Ph.D. degree in 1984 in applied microbiology at the University of Saskatchewan (thesis: "Ethanol tolerance of brewers yeast in high gravity brewing"). Following 2 years as a NATO postdoctoral scientist at Carlsberg Laboratories in Copenhagen researching yeast chromosome fingerprinting and diacetyl production by lager yeasts, he returned to the University of Saskatchewan as an assistant professor in the Food Biotechnology Department (1986–1987). Since then, Greg has been employed as a senior research scientist with Anheuser-Busch in St. Louis (1987–1991), senior project leader in charge of the Strain Development Laboratory at Red Star Yeast and Products in Milwaukee (1991–1992), and senior director responsible for the Corporate Laboratories at the Stroh Brewery Company in Detroit (1992–1999). Greg joined Coors Brewing Company in April 1999 and, since that time, has served in the capacities of director of corporate quality assurance (1999–2003), director of brewing R&D (2002–2003), and since 5/04, director of brewing services.

O-79

The Fuel Alcohol Industry: She's Younger, She's Bigger, but Is She Wiser?

W. M. INGLEDEW

University of Saskatchewan

Although the alcohol fermentation industry is mature and has had a long history, the brewing industry has led in technology, education, and the dissemination of the science behind the process. Winery and distillery technology have also advanced. The fuel alcohol industry is not new since it was practiced seriously after the production of the automobile, in times of war, and for industrial alcohol manufacture. The process was not economic, however, since the late 1920s, when low-priced petroleum became available. Since the mid 1970s, production of fermentation alcohol for automobile fuel has steadily increased, and the technology has developed quickly. Because of its youth, the fuel industry has not had the same time to mature through research, education, and technological advances. This presentation will inform brewers and allied industries of the extent of the fuel alcohol industry, its problems and its successes.

Mike Ingledew received his B.Sc. and Ph.D. (1969, microbiology/biochemistry) degrees at the University of British Columbia and completed postdoctoral studies (1970, cellular biology) at CSIC in Madrid. He has conducted research in brewing, winery, distillery, and fuel alcohol technology since 1971 at his University of Saskatchewan laboratory. He received the Eric Kneen Memorial Award (1994) from ASBC, the Award of Recognition (1996) for outstanding services to brewing from MBAA (Western Canada), and the International Biotechnology Medal of Excellence (1999) for work advancing the biochemistry of yeast in alcohol production from Alltech Biotechnology Inc. He has published more than 150 research papers and has had a very long commitment to education courses in brewing and fuel alcohol production. He is a past editor-in-chief of the Journal of the ASBC. He consults with the fuel alcohol and distilling industry worldwide.

O-80

Aroma-Active Ester Formation in Brewer's Yeast: What, How, Where, Why, and How to Control It?

KEVIN J. VERSTREPEN (1,2), Jean-Pierre Dufour (3), Isak S. Pretorius (4), Johan M. Thevelein (5), and Freddy R. Delvaux (1)
(1) Centre for Malting and Brewing Science, K.U. Leuven, Belgium; (2) M.I.T. Whitehead Institute, Cambridge, MA; (3) Department of Food Science, University of Otago, New Zealand; (4) The Australian Wine Research Institute, Adelaide, Australia; (5) VIB Department for Molecular Microbiology, K.U. Leuven, Belgium

Fermenting yeast cells produce a wide variety of volatile aroma-active esters that are of great importance for beer flavor. In high-gravity beer fermentations, performed in tall cylindrical vessels, the beer ester balance is often suboptimal, resulting in a clear decrease in beer quality. The aim of this work was to gain more insight into the biochemical aspects of the formation of aroma-active esters and to investigate how brewers can control ester synthesis. First, the role and relative importance of the known *Saccharomyces cerevisiae* alcohol acetyl transferases, Atf1p, Atf2p, and Lg-Atf1p, were investigated. The respective genes were deleted and overexpressed in a laboratory and a commercial brewing strain. Subsequently, the ester formation of the transformants was monitored using headspace gas chromatography (HS-GC) and gas chromatography combined with mass spectroscopy (GC-MS). It was found that the expression levels of *ATF1* and, to a lesser extent, *ATF2* greatly affect the production of ethyl acetate and isoamyl acetate during small-scale wort fermentations. GC-MS analysis showed that Atf1p and Atf2p are also responsible for the formation of a broad range of less known acetate esters. Northern blot analyses of *ATF1* showed that this gene was rapidly induced by addition of glucose to anaerobically grown, carbon-starved cells. Further investigation showed that the Ras/cAMP/PKA signalling pathway is responsible for this regulation. Furthermore, nitrogen was needed in the growth medium in order to maintain the *ATF1* expression. In addition to nutrient regulation, *ATF1* expression levels were also affected by heat and ethanol stress. These findings explain the effect of medium composition on volatile ester synthesis in industrial fermentations. More specifically, it was shown that the use of high-glucose and high-nitrogen fermentation media enhances the *ATF1* expression levels, which results in higher ester synthase activities and an increased formation of acetate esters. In order to reveal the subcellular localization of Atf1p and unravel the physiological role of this protein, *ATF1::GFP* fusion constructs were overexpressed in brewer's yeast. UV fluorescence microscopy combined with cellular fractionation revealed that Atf1p is localized in lipid particles. This suggests that Atf1p has a specific role in the lipid and/or sterol metabolism that takes place in these particles. Taken together, our study reveals the importance of Atf1p for the synthesis of acetate esters and explains how brewers control the formation of these important flavors by adapting specific fermentation parameters.

Kevin Verstrepren graduated in biological engineering, option gene technology, from the Catholic University of Leuven, Belgium. For his M.Sc. thesis, he joined the group of Prof. Sakkie Pretorius at the University of Stellenbosch to study the use of genetic modification to improve the flocculation behavior of brewer's yeast. A year later, he returned to Belgium to start a Ph.D. program in the group of Prof. Delvaux at the Center for Malting and Brewing Science and the group of Johan Thevelein, Laboratory for Molecular Cell Biology. Between 1999 and 2003, Kevin investigated flavor-active ester formation in brewer's yeast. After earning his Ph.D. degree, Kevin was appointed as a post-

doctoral fellow in the laboratory of Prof. Gerald Fink at M.I.T. in Cambridge, MA. He now studies the genetic variability and regulation of the yeast flocculation genes. He also serves as a group leader at the Centre for Malting and Brewing Science, where he coordinates a research project into the synthesis of volatile ethyl esters in yeast. Kevin is author of several publications and regularly serves as a reviewer for different scientific journals and financing institutes. He is a member of the American Society for Microbiology, the EBC fermentation subgroup, and the Royal Belgian Association of Brewing Science Alumni. He was awarded several prizes and was recently named an honorary fellow of the Hoover Foundation.

O-81

Quality Improvement in Continuous Main Fermentation with Immobilized Yeast

ANDREAS LUDWIG and Karl Wackerbauer
University of Technology of Berlin, Chair of Brewing Science, FBM der VLB Berlin

For several years, efforts have been made to define processes for continuous fermentation and maturation with immobilized yeast. Systems for continuous maturation have been introduced and applied at an industrial scale, while applications for continuous main fermentation using immobilization are still in the research stage. One important reason for this fact is that the course of process and the quality of the final product are not stable during reactor operation; constant changes of both are observable. On the other hand, continuous main fermentation with immobilized yeast provides a lot of advantages, such as a considerably increased fermentation performance per volume bioreactor; smaller buffer capacities needed; and due to long-term operation, less expenses for yeast propagation, yeast management, and cleaning compared with batch fermentation. In our investigation, we evaluated different technologies imaginable for an application with immobilized yeast. The main task was to improve the long-term stability of the continuous main fermentation compared with previous experiments. For this purpose, we used three different reactor systems, the defined aeration of the reactors, and a selection of carrier materials that appeared to be promising for our goals in terms of process and product quality. We had the unique chance to evaluate different technological options without being fixed to only one system. Besides the aspects of process technology, we included investigations that refer to the controllability of biological systems using simply measurable or adjustable physical and chemical quantities. We developed a controller software installed on a process control system, which was connected to the pilot reactor. As process quantities, pH value and reactor aeration rate were used and adapted by the controller. The controllability of the continuous process was also investigated, with an emphasis on a constant situation regarding the process as well as the product. It is possible to improve long-term stability significantly by a toolset of technological measures.

Andreas Ludwig had an apprenticeship in brewing (Privatbrauerei A. Rolinck, Steinfurt, Germany; 1990–1992), studied brewing science at the University of Technology of Berlin (TU Berlin; 1992–1997), and was a process engineer for R&D and plant commissioning (EUWA Water Treatment Plants, Gaertringen, Germany; 1997–1999). From 1999 to spring 2004, Andreas was a scientific assistant with teaching duties at the Chair of Brewing Science of TU Berlin, had a research project and Ph.D. thesis on "Main fermentation with immobilized yeast", was head of pilot brewery and pilot plant of TUB/VLB Berlin, was a consultant in brewing technology for VLB Berlin, and was a lecturer in the Brewing School of VLB Berlin for "Energy Technology" and "Process Control Systems". Since June 2004, Andreas has been head of the Central Laboratory, Radeberger Gruppe AG Breweries, Fankfurt, Germany.

O-82

The New Method for Drying Lager Yeasts

TAKAAKI IZUMI, Hideko Yomo, Katsumi Oshita, Hitoshi Matsubara, Nobuyuki Fukui, and Yasutsugu Kawasaki
Suntory Ltd.

Dry yeasts have been widely used for making bread and brewing wine. Dry yeasts for brewing lager beer namely, dry lager yeasts, should be also very available for yeast storage and management of beer production. But there aren't any kinds of dry yeasts on the market employed by our brewery. And even when dry lager yeasts are produced by conventional

method, i.e., drying aerobically cultured yeasts, the beers produced by using these dry yeasts have unfavorable qualities. For example, the beers contain few esters and lower sulfur dioxide. So we tried to develop a new method of drying our employing lager yeasts by maintaining their fermentation property. We noticed that intracellular trehalose increases resistance to drying in yeast. Furthermore, we anticipated that sugar alcohols are capable of exerting an effect similar to that of trehalose. From these points of view, we developed our original method to make lager yeasts recovered from the beer fermentation step incorporate extracellular trehalose and sugar alcohols. By using this method, we could give our lager yeasts resistance to drying without changing their fermentation property. As a result, it was found that these dry lager yeasts maintained high activity and exhibited a favorable fermentation property. And we confirmed that the beers produced by using these improved dry lager yeasts showed similar fermentation activity and quality to the beers produced by not using dried yeasts.

Takaaki Izumi received an M.S. degree in biotechnology from Osaka University. He began employment with Suntory Ltd. in 2000 as a researcher in the Institute for Fundamental Research. Since 2002, he has worked at the Institute for Beer Development. He has been studying the development of brewing technology.

O-83

Control of the Yeast Propagation Process—How to Optimize Oxygen Supply and Minimize Stress

OLAU NIELSEN

Alfa Laval Scandi Brew

More than 100 years after its introduction, the yeast propagation process is still not fully understood because of the yeast's facultative nature. In the brewing industry, the desire is to use yeast with good fermenting characteristics but also with the ability to grow fast while maintaining these characteristics during propagation. The problem with growth is that the catabolite repression—the Crabtree effect—limits the yeast's ability to take up oxygen during yeast propagation in wort. Furthermore, in later years, we have seen a growing awareness that certain factors during propagation may stress the yeast and thus influence yeast vitality and beer quality. The stress factors during propagation are closely related to the aeration method. The fundamental questions are, therefore, how and how long to aerate plus how much oxygen to supply? In order to answer these questions, a research study has been carried out. The basis for the study was a newly developed aeration device designed as an off-center agitator with a hollow shaft for air supply. The tests were carried out in a 10-hL test propagator and later in different full-scale propagators in breweries with net volumes ranging from 20 to 160 hL. The intention was to try to detect any signs of stress as a result of the use of the agitator and to find the optimum aeration profile during the propagation. Using a 160-hL net propagator in the most recent experiments, the outlet gas from the propagator was monitored and the oxygen content and gas volume were compared with cell growth and yield factors. As this control is very informative, but also quite complicated, a more simple control was also sought, and some valuable and easily applied methods were developed. The main findings so far are the following. No stress can be detected as a result of the use of the agitator. The benefit of a high concentration of dissolved oxygen during propagation is limited. On the other hand, it seems that oxidative stress is a smaller problem than carbon dioxide stress, so the aeration must be sufficient to assure a low concentration of carbon dioxide in the propagator. By means of monitoring the amount of gas in the outlet from the propagator, it is also possible to determine when the aerobic metabolism stops. A more simple, but equally reliable, way to monitor this is by measuring the yield factor as number of million cells produced per degree Plato consumed. Low yield factor means no aerobic activity. An even more simple, but not so reliable, method is to observe the foam formation. High foam means big amounts of carbon dioxide caused by a high Plato consumption. High Plato consumption means anaerobic metabolism—and no aerobic growth.

Olau Nielsen was born in 1951 and graduated from the Technical University of Denmark with a M.Sc. degree in biochemical engineering. Olau worked for many years in other bioengineering fields before joining Scandi Brew in 1996. In Scandi Brew, Olau holds a position as sales & technology manager for yeast, which is the core product of the company (today Alfa Laval Scandi Brew). The work has concentrated around developing aeration aggregates for yeast propagation plants and mixers

for yeast storage plants with a focus on efficiency and low stress conditions. Olau's latest work has involved propagation tests verifying the influence of the Crabtree effect on yeast propagation and investigating measurable stress as a result of mechanical impact on propagated yeast caused by agitation and aeration. Olau's previous work was published at IGB 2003 in Livingstone, EBC 2003 in Dublin, and 4th BYFPC 2003 in Oxford. Planned work involves optimal oxygen supply, oxygen balance and measurable oxidative stress during propagation, and further research into yeast stress conditions during storage.

O-84

Different Physiological Marker to Monitor Yeast Propagation and Fermentation by Flow Cytometry

KARL-JOSEF HUTTER

Eichbaum Brauereien AG, Mannheim, Germany

Flow cytometry provides a rapid and accurate means to monitor the physiological state of yeast cells throughout the fermentation process. This technique allows a near-real time monitoring of the physiological state of yeast populations in industrial fermentations. In the past, we have developed several flow cytometric methods to assess key parameters of the physiological condition of yeast cells, e.g., DNA, glycogen, trehalose, and neutral lipid contents, and proteinase A activity. In common brewing practice, yeast populations are often pitched in the wort in their quiescent (G0) or G1 phase of the cell cycle. These yeast populations need several hours to reach their exponential growth phase with most of the cells in G2 or mitoses and, hence, fermentation to target gravity takes longer. We optimized the propagation of lager yeast by assessing the cell cycle of the yeast population using flow cytometry. This enabled us to establish an improved pitching regime in which the yeast was inoculated in their exponential growth phase, leading to significantly shortened fermentation durations. Furthermore, we monitored the physiological state of lager yeast during fermentation of 18° Plato wort. We found significant variations in the physiological parameters. The increase of the number of exhausted and/or dead yeast cells was indicated by the appearance of yeast subpopulations with different glycogen or trehalose contents. The aim of this contribution is to demonstrate the multiple possibilities of flow cytometric acquisition in industrial processes.

Karl-Josef Hutter was born in 1943 in Dietfurt/Altmühl, Bavaria. Karl-Josef's native country is the Federal Republic of Germany. Karl-Josef studied brewery technology at the TU-Berlin (VLB) from 1965 to 1970 and graduated in 1974. Karl-Josef had vocational training from 1960 to 1962 at Brauerei Frankenheim, Düsseldorf. From 1970 to 1979, Karl-Josef was a scientist at Fraunhofer-Gesellschaft, and since 1979, has been a scientist at the German Cancer Research Center, Heidelberg. Karl-Josef has maintained lectureships at the University of Heidelberg (1985–1992), University of Hohenheim (1994–1999), Fachhochschule Mannheim (1995–present), and TU-Dresden (1999–present).

O-85

Stress and the Regulation of Brewing Yeast Flocculation

KATHERINE A. SMART, Cheryl L. Jenkins, Steve Davy, Jessica Leclair, and Stephen Lawrence
Oxford Brookes University

On completion of brewing fermentations, yeast biomass is harvested (cropped) from the fermentation vessel and stored until it is required for use in subsequent fermentations. This recycling process is known as serial repitching. The yeast-harvesting procedure relies on the capacity of the brewing yeast population to aggregate into clumps at the end of fermentation and readily sediment. This process is known as flocculation and is critical for yeast collection and beer clarification. Aberrant flocculation can result in the formation of either an over- or partially fermented product that cannot be processed to final product, leading to significant inefficiencies for the brewer. We have recently demonstrated that the flocculation potential of brewing yeast populations is a function of the number of fermentations the yeast has conducted. Analyses of yeast populations derived from full-scale production fermentation and storage vessels suggest that serial repitching enhances flocculation performance. In part, this is due to inadvertent selection of subpopulations during harvesting; however, serial repitching also results in exposure to a number of stresses that affect the expression of key cell wall mannoproteins that permit flocculation to occur. It has been established that critical stresses occur at distinct stages of the recycling process. Indeed, during

fermentation, yeast cells are exposed initially to oxidative stress followed by anaerobiosis ethanol toxicity and low pH. During storage between fermentations, yeast cells additionally become starved and are exposed to cold shock. The impact of three key stresses, the shift from aerobiosis to anaerobiosis, a reduction in pH, and the application of cold shock, on the expression of the CWP gene family that encodes cell wall mannoproteins is considered. The relationship between CWP gene expression and the potential of the yeast cell wall to flocculate and express cell surface physical properties is demonstrated. The functional phenotypes associated with the expression of CWP genes will be discussed and a model describing their potential role in flocculation will be suggested.

Katherine Smart completed a B.Sc. degree (Hons) in biological sciences at Nottingham University and was awarded the Rainbow Research Scholarship to complete a Ph.D. degree in brewing yeast physiology at Bass Brewers, Burton-on-Trent. She then moved to Cambridge University to take up an appointment as research fellow in the Department of Plant Sciences, where she worked on bioactive surfaces, biofouling, and bacterial contamination of beverages. In 1992, Katherine became a lecturer and then senior lecturer in microbiology and fermentation at Oxford Brookes University. Now the Scottish Courage Reader in Brewing Science and a fellow of the Institute and Guild of Brewing, Katherine holds a Royal Society Industrial Fellowship. Katherine is a member of the several societies and has served on society committees and journal editorial boards. She is chair of the Institute and Guild of Brewing International Section and the American Society of Brewing Chemists' international director.

TECHNICAL SESSION XIV: Health and HACCP

Moderator: Rob Maruyama

Robert Maruyama graduated from the University of Colorado in Boulder with a B.A. degree in molecular, cellular developmental biology and received an M.S. degree in environmental science and engineering from the Colorado School of Mines. He joined Coors in 1980. During his tenure at Coors, Rob was responsible for analytical methods development using gas chromatography and high-performance liquid chromatography, development of laboratory automation applications, and analytical project management. In 1994, he was named laboratory supervisor, in which he was responsible for the organic laboratory operations, which supported environmental control and container manufacturing. Rob was promoted to research and quality assurance laboratory manager in 1995, in which he was responsible for managing the analytical laboratory that supports brewing research and development and corporate quality assurance. In 1999, Rob was promoted to the position of director of product quality in the Golden Brewery Business Unit, where he is responsible for the QC functions in malting, brewing, packaging & logistics operations. In addition to his role in quality, Rob assumed the responsibilities for Golden's environmental health and safety in 2000. Rob is a member of the ASBC and ACS and has made presentations and posters to ASBC and AOAC. Rob has served ASBC in many capacities: an active subcommittee participant; chair of a number of technical subcommittees, including the Coordination of New and Alternate Methods, publications chair; and president in 2001. Rob is also the WBC 2004 Planning Committee cochair.

O-86

WITHDRAWN

O-87

Beer and Folates

CAROLINE WALKER, Christopher Booer, Robert Smith, Benn Kerr, and Andrew Faulkner
Brewing Research International

Functional, health-enhancing foods are a hot topic. Consumers are avidly searching for foods that are natural sources of nutrients and vitamins, and nutritionists are encouraging us to improve our diets. One particular area of concern is that folate (vitamin B9) is often lacking in Western diets, which may put the population at an increased risk of chronic disease, including cardiovascular disease and cancer. It is, therefore, a public health issue to find out which foods can make a significant contribution to folate intake in the diet. With this problem in mind, during the last 4 years, we have been funded by the EU and the Home Grown Cereals Authority

to look at the folate content of malt and beer. From a survey of commercial samples, we have shown that folate levels in malt range between 2 and 4 mg/kg and are several times higher than those found in barley. Using small-scale and pilot-scale trials, we found that the folate content in the grain was increasing during malting, and this rise was dependent upon conditions. For example, high-diastatic-potential malts had higher folate contents than did ale or lager malts, whereas crystal and roasted malts had much lower levels. However, malting conditions were not the only important factor influencing folate content. Field trials showed that both growth conditions and barley variety influenced the folate content of the finished malt, raising the possibility of directed breeding programs for a 'high-folate' trait. The folate in malt is carried through to the beer during brewing. From our survey of international beers, we found folate contents ranging from 30 to 150 µg/L. In general, beers with higher folate content also had higher alcohol content, probably reflecting malt levels in the grist. Results from pilot commercial brewing trials revealed that folate recovery was poor during mashing but good through the rest of the brewhouse. Laboratory-scale work suggested that folate recovery could be optimized during mashing by adjustment of temperature and liquor/grist ratio. Primary fermentation did not affect folate levels, whereas secondary fermentation, such as that carried out during bottle conditioning, seemed to enhance folate content, possibly due to the physiological condition of the yeast during this process. From our data, we can conclude that, for those who consume moderate amounts of beer regularly, folate intake from beer may be in the order of 10–20% of the daily intake. This is a very high contribution from any one foodstuff and is of dietary significance in beer-drinking populations. Although the levels of folate in beer are significant, our data suggest that, by careful selection of raw materials and some changes in the brewing process, beers with a higher folate content could be produced.

Dr. Caroline Walker holds a degree and doctorate in biochemistry from the University of Bristol. She is manager of the health program at BRi and acts as a consultant on beer and health for BRi's international membership. Along with leading BRi's research in this area, Caroline plays a key role in communication and, as a member of the British Guild of Beer Writers, publishes articles on all aspects of health and brewing. Caroline is also the head of process at BRi, and her group covers research in the areas of microbiology, engineering, molecular biology, and fermentation.

O-88

About Beer and Celiac Disease

MICHAEL J. LEWIS (1) and Charles H. Halsted, M.D. (2)

(1) Department of Food Science and Technology, University of California;

(2) Department of Internal Medicine (Gastroenterology and Clinical Nutrition), University of California

Celiac disease, also called celiac sprue, is an auto-immune disease in which a reaction to a sequence of amino acids in prolamines, especially gluten, causes deformation of absorptive villae of the small intestine. As a result, nutrients are poorly absorbed. Children fail to thrive and, in the adult-onset form of the disease, severe weight loss is a characteristic, with malaise, loss of calcium from bones, iron deficiency, dermatitis, potential loss of night vision, and increased chance of diabetes and certain cancers. The disease affects about 1% of people, depending on the population surveyed, but the disease is undoubtedly underdiagnosed in the U.S.A. Although gluten occurs at its highest concentration in wheat-based products, which celiacs must assiduously avoid, other prolamine-containing grains, including barley and malt, must also be excluded. Although beers contain very small amounts of gluten fragments (as currently measured), all beers are banished from the diets of celiacs by the standard that they are not made from gluten-free raw materials. Although there is a possibility that a beerlike drink could be made from grains other than malt, celiacs might prefer to resign themselves to cider, wine, spirits, and RTDs. Considerable progress has been made in identifying the specific amino acid sequence and protein fragments of gluten that trigger the immune reaction, and physicians have begun to consider ways to alleviate the disease other than by strict and life-long dietary control. One potential method (among several target approaches) is to eliminate the offending protein fragments during food manufacture. This is clearly well suited to beer and brewing, because much protein is eliminated during malting and brewing and manipulation of grain proteins is well understood by brewers as a central part of normal processing. This paper will explore these possibilities. Eating "gluten free" is becoming popular among food

aficionados and faddists, perhaps as a natural extension of the famous Atkins' diet. As the brewing industry has successfully harnessed the ultra-low carbohydrate content of some beers to this dietary choice, so it may prosper from a developing "eat-gluten-free" movement.

Prof. Michael J. Lewis conducted the program in brewing science at the University of California, Davis for 40 years and is now emeritus professor. He is a fellow of the Institute and Guild of Brewing (London), is a life member of the American Society of Brewing Chemists, and received the prestigious Award of Merit of the Master Brewers Association of the Americas in 1986. The second edition of his book Brewing has been well received. In 1990, he won the esteemed Distinguished Teaching Award of the University of California. Dr. Lewis teaches several specialized courses through University of California Extension (UNEX), including the accredited 4-month Masterbrewers Program leading to a professional qualification in brewing science and engineering (the Associate Membership Examination of the Institute and Guild of Brewing), and a short (8-week) form of this program called the Professional Brewers Certificate Program. Graduates of Dr. Lewis' programs are well represented in large and small North American breweries as well as abroad. Dr. Lewis earned his Ph.D. degree in microbiology and biochemistry at the University of Birmingham (England) and the British School of Malting and Brewing. Dr. Lewis has also served the University as assistant vice-chancellor of academic affairs and associate dean of the College of Agriculture.

O-89

HACCP Accreditation at Labatt-Interbrew North America—Corporate and Brewery Perspectives

JESSICA HUDALE (1) and TERRANCE M. DOWHANICK (2)
(1) Latrobe Brewing Company, LLC (Labatt-Interbrew), Latrobe, PA; (2) Labatt-Interbrew North America, London, ON, Canada

In March 2000, the decision was made by senior corporate management at Labatt-Interbrew to have each of its nine breweries in North America HACCP (Hazard Analysis Critical Control Point) accredited before the end of 2004. The first part of this presentation will focus on the challenges faced and strategies employed from a corporate perspective in achieving this goal. The second part of this presentation will focus on HACCP at the brewery level, as experienced by the Latrobe Brewery. Discussion will focus on the internal technical challenges encountered and the overall culture change that led to the successful implementation of HACCP, making Latrobe the first third-party HACCP-accredited brewery in North America in January 2003.

Jessica Hudale received a B.S. degree in microbiology from The Pennsylvania State University in State College, PA. She began her career with Latrobe Brewing Company, LLC in October 2001 as the HACCP coordinator. In January 2003, Latrobe Brewing Company, LLC became the first brewery in North America to achieve HACCP accreditation by a third-party auditing body. Since June 2003, Jessica has functioned as compliance manager, focusing on food safety as well as environmental health and safety. Jessica served the MBAA on the local level as secretary/treasurer from January–December 2003 and is currently serving as the vice president of her local chapter. She has been a guest speaker at a local MBAA meeting and also presented a talk on HACCP at the Northern California Annual MBAA Technical Conference at the Sierra Nevada Brewery in June 2003. Terrance M. Dowhanick, Ph.D. (Carleton University), B.Sc. (York University), began his career at Labatt in 1982 in brewing R&D. From 1984 to 1988, he served as the Labatt visiting scientist for genetic biotechnology at the National Research Council in Ottawa. He holds one patent and has authored/coauthored more than 40 research papers and review articles in the areas of yeast gene expression and microbial diagnostics for the brewing industry. From 1996 to 1998, he was the quality technical manager for the Labatt-Interbrew London Brewery, and from 1998 to 1999, he was science resource manager for technology development as well as quality assurance manager. Since 2000, he has been responsible for quality assurance, product integrity, and food safety for Labatt-Interbrew throughout North America. Terry is an alumnus of the Ivey Executive Business School, member of the Institute and Guild of Brewing, past chair of education for the Master Brewers Association of the Americas, has served on the Board of Directors for the American Society of Brewing Chemists, and is the chair of the Technical Committee for the Brewers of Canada.

O-90

Effective Strategies in Implementing HACCP in San Miguel Breweries

ARNULFO Z. SENIRES (1) and Niceforo V. Alegado (2)
(1) San Miguel Beer Division Quality Assurance; (2) San Miguel Davao Brewery

Two years ago, our technical department saw the need for an enhanced product safety assurance program and chose to implement Hazard Analysis and Critical Control Points (HACCP); it also anticipated possible regulatory requirements in the local and export market San Miguel serves. Strategies were planned to ensure fast and effective implementation of HACCP. It was expected that voluminous documentation and difficulties in writing the HACCP plans, as well as in the implementation, would be encountered since the process of brewing is more complex than in other food industries where HACCP is working well. HACCP-based Malt Beverage Safety and Quality Policies and Guidelines were developed by Beer Division Quality Assurance and endorsed by Beer Division Management. Initial risk assessments of raw materials, process operations, equipment, and machinery were done by Beer Division Quality Assurance. Generic models of brewing-process flowcharts and HACCP plans were also drawn. To gain hands-on experience and expertise, a brewery in southern Philippines, Davao Brewery, was chosen as the model and experimental plant for HACCP and enhanced current Good Manufacturing Practices (cGMP). On-site lectures and workshops on HACCP and cGMP were conducted by Brewing Quality Assurance to the HACCP-cGMP Team of the brewery. During the workshops, the participants were taught how to determine and address noncompliance to cGMP, carefully meld new structures and equipment (which are the requirements of cGMP) with present facilities, conduct risk assessments, make an HACCP plan, identify and confirm the Critical Control Points (CCPs), monitor the CCPs, and conduct an HACCP-cGMP audit. In the course of the workshops, the generic models of flowcharts and HACCP plans were revised in accordance to the brewery's process operations and practices. The participants echoed what they learned to all other employees as well as to the management team of the brewery. Lectures and workshops were then extended to the other breweries, four in the Philippines, one in Hong Kong, and three in China, while those in Indonesia, Vietnam, and Australia have been scheduled for the current year. After a year of preparation and implementation, Davao Brewery has undergone external and third-party HACCP-cGMP audits. Other breweries are scheduled for similar audits. All the breweries where HACCP and cGMP concepts and practices were cascaded are now implementing the systems despite the lean manning.

Arnulfo Z. Senires has been a quality assurance specialist of San Miguel Beer Division Quality Assurance since the latter part of 2000. He holds a B.S. degree in chemistry and a M.Sc. degree in chemistry from the Far Eastern University and the University of Santo Tomas, respectively, both in Manila. In 1974, he started working in San Miguel's Aviles Brewery and then moved to Polo Brewery in 1976, until 1992, when he was assigned to set up San Miguel's Beer Central Analytical Laboratory, making it the first ISO 17025-accredited private manufacturing laboratory in the Philippines.

O-91

Taking Complexity and Cost Out of the Brewing Industry Supply Chain

CHRIS WALLACE (1) and Barbara Roos (2)
(1) Scottish Courage Ltd.; (2) Agilisys

This presentation will look at how the beer industry can drive supply chain performance improvements while being constrained by a unique set of planning and scheduling challenges. Based specifically on the experiences of one of the world's largest beer manufacturers, Scottish Courage, and indirectly on the practices of other known brewers, this presentation will reveal how technology can enable greater supply chain efficiencies in the brewing industry. As beer consumption increases worldwide, brewers are fighting for market share and are challenged to provide a more diversified product line. To stay competitive, brewers must produce the right product in the right quantities at the right time, while minimizing production and distribution costs. This is not an easy task when challenged by some of the most unique supply chain constraints, such as tank scheduling, complex piping networks, buffers, moving bottlenecks, and variable changeovers. To further the complexity, the expansion of contract brewing puts even

greater pressure on brewers to more efficiently utilize existing capacity to produce more beer. Some of the largest beer manufacturers in the world rely on Agilisys solutions to enable more efficient beer production. Scottish Courage, leading brewer in the U.K. and one of the largest in Europe, uses Agilisys Advanced Scheduling technology to manage production costs by optimizing their plant and keeping safety stocks in balance. With production lines producing over 10 million barrels of beer each year and contract brewing driving more SKUs, Scottish Courage relies on Agilisys for its scheduling success. They now have a clear picture of which beer to produce in which tanks and in which sequence to make best use of their resources to satisfy customer demand at the lowest possible production cost. They have reduced scheduling time and production interruptions, have improved synchronization from brewing to packaging, and now have better visibility of mature beer availability. And, they now benefit from centralized information that allows complete optimization of their multisite U.K. operations. Agilisys Advanced Planning technology also plays a key part in optimizing beer production by balancing supply and demand while considering material and logistical costs, manufacturing capacities, and other constraints to determine the most feasible beer production plan. Other well-known beer manufacturers, such as SAB Miller, Molson, Grolsch, Fosters, and Heineken, are taking advantage of Agilisys brewery-focused solutions. Benefits that Agilisys customers are achieving include reduced production and distribution costs, reduced efforts and time for scheduling and planning a brewery, improved throughput through all processes, “what-if” investment analysis, improved service, increased plant flexibility, and more.

Chris Wallace received his degree in microbiology in 1985. He began his employment with Express Dairies and was later given the position of laboratory analyst. Chris then moved to The Stag Brewery at Mortlake, London, and 2 years later was promoted to process controller, initially as part of the commissioning team for a £22 million lager process block. Chris then moved to become a canning manager, first at Iselworth West London, and then as part of the team that moved and reinstalled the line at The Courage Berkshire Brewery, Reading. He was in charge of the day-to-day running of two high-speed canning lines. Chris then moved to Scottish Courage's Royal Brewery in 1996 as site planning manager, responsible for the day-to-day planning and forecasting of a site producing 3.0 million hL per annum. He moved into a project role in January 2000 within logistics to realign the supply chain to the changing needs of the customer. During this time, he acted as project leader in implementing Agilisys Advanced Scheduling at the five Scottish Courage sites.

O-92

Popular Diets and the Nature of Beer Carbohydrates

NATHANIEL J. DAVIS
Anheuser-Busch, Inc., St. Louis, MO

Current popular carbohydrate-focused diets are more sophisticated and complex than simply counting carbohydrates and reducing overall carbohydrate intake. They include nutritional concepts such as the Glycemic Index. They discuss how different carbohydrates behave and are metabolized in the body and how they interact with other dietary components. The nature of the carbohydrate content is now often considered at least as important as the level when choosing food within the context of some of these diets. Carbohydrates and the foods that contain them are ranked and categorized as “good” or “bad” based on these concepts, and many people are making their food and beverage choices, in part, according to advice from these books. Many of these books contain serious technical errors about the nature of the carbohydrates in beer. They are neither brand- nor style-specific and, therefore, the misinformation is applied to all beer. Some carbohydrate-focused diet books incorrectly characterize beer as being high in sugar (particularly maltose); as having a high Glycemic Index, therefore, as being more problematic than other alcoholic beverages of similar calorie levels; and even as specifically causing the deposition of fat in the abdomen due to the presumed nature of beer's carbohydrate profile. This article reviews the errors regarding beer in popular carbohydrate-focused diets and the metabolic concepts that underlie these diets. How beer relates to these concepts and the true nature of carbohydrates in a variety of beer styles is reviewed.

Nathaniel J. Davis is a staff brewmaster of corporate brewing at Anheuser-Busch, Inc. (A-BI), St. Louis, MO. As a staff brewmaster, Nathaniel's primary responsibility is to develop new products. Nathaniel

is responsible for developing new product recipes and brewing procedures, planning production processes, and helping to oversee the brewing of new and specialty products at Anheuser-Busch. Nathaniel works closely with A-BI's Brand Marketing group to ensure that new products match both marketplace trends and consumer expectations. In the past year, he oversaw development of the company's new products, including Anheuser World Select, Bare Knuckle Stout, and ZiegenLight. Prior to his current position, Nathaniel served on the brewmaster's staff at Anheuser-Busch's Fort Collins, CO, brewery. Nathaniel is a member of the Master Brewers Association of the Americas and the Institute and Guild of Brewing. Nathaniel was born in Kingston, Ontario, Canada. He is a graduate of the Master Brewers Program at the University of California at Davis. He also holds a bachelor of science degree in microbiology and immunology from McGill University in Montreal, Canada.

O-93

Pilot-Scale Investigations into the Production of Filtered Beers Rich in Xanthohumol

MARTIN BIENDL
Hopsteiner - Hallertauer Hopfenveredelungsgesellschaft

In the past few years, many reports on potential health effects of the hop compound xanthohumol have been published. Especially promising seems to be its cancer chemopreventive activity. Animal studies to investigate its bioavailability and metabolism are currently ongoing. In dried hop cones, xanthohumol is present at a concentration of up to 1%. Its ratio to the alpha-acids is a varietal characteristic and ranges from 0.02 to 0.1. During hop processing, xanthohumol is almost completely recovered in pellets and ethanol extract but not in carbon dioxide extract. During wort boiling, it is converted to isoxanthohumol. This compound also shows positive health effects, although it seems to be less effective than the nonisomerized form. Due to the isomerization process, commercial hopping results in levels of up to about 2.5 mg/L isoxanthohumol in filtered beers, whereas the xanthohumol content is generally lower than 0.2 mg/L. However, during the production of stouts and porters, the isomerization is partly inhibited. By using a commercial ethanol extract of the variety Spalter Select, a concentration of about 1 mg/L xanthohumol could be achieved in a stout after polishing filtration. This concentration was tripled by using a recently developed hop product. This new product is the residue of a secondary extraction of ethanol pure resin extract with supercritical carbon dioxide. It has a ratio of xanthohumol to alpha-acids greater than 1. Other than using the xanthohumol-enriched hop product, no technological changes were necessary for achieving this high concentration of xanthohumol in the stout. As an alternative technology to transfer nonisomerized xanthohumol into beer, addition after fermentation was also investigated. For this application, xanthohumol with a purity above 80% was isolated from ethanol pure resin extract. The purified xanthohumol was added as an ethanolic solution to cold beer before filtration. Depending on the degree of filtration, concentrations in the range of about 1 to 3 mg/L were achieved. Higher concentrations could not be achieved due to the low solubility of xanthohumol in beer.

Martin Biendl received a Ph.D. degree in organic chemistry from Regensburg University in 1990. Since then, he has been employed as head of research and development at the Hopsteiner - Hallertauer Hopfenveredelungsgesellschaft in Mainburg, Germany. Since 1996, he has been section manager of the R&D/Quality Assurance Department of this company. He is a representative of the International Hop Industry Cooperation (IHIC) in the EBC Analysis Committee.

TECHNICAL SESSION XV: Malting/Mashing

Moderator: Rob McCaig

Rob McCaig has more than 22 years of brewing industry experience with Molson Breweries. Starting his career in 1981 with Molson in Quebec, Rob has held a number of positions including research microbiologist, brewer, corporate brewer, and brewmaster. During his time with Molson, he published more than 20 technical papers and was responsible for developing more than 50 new beer brands. In February of 2003, he left Molson to take the position of managing director and director of brewing for the Canadian Malting Barley Technical Centre (CMBTC) in Winnipeg. Rob is a member of the American Society of Brewing Chemists (ASBC), serving as both local chair and as president of the national ASBC. He is also a member of the Master Brewers Association of the Americas and the

Institute and Guild of Brewing. He has a master of science degree in applied microbiology from the University of Guelph. He resides in Winnipeg with his wife Louise and two sons, Alec and Ian, where he still plays hockey weekly, flyfishes with his ASBC ROR group, and helps coach both boys in hockey.

O-94

Parameters Influencing the Mash Filterability in the Brewing Process

ROBERT BRAEKELEIRS (1) and Rafael Tigel Gil (2)

(1) MEURA s.a., Belgium; (2) MEURA Technologies, Belgium

The filterability of the mash in a brewhouse equipped with a lauter tun or a thin-bed filter is influenced by different parameters: • Viscosity of the mash enhanced by the variety of malt due to the under- or overmodification, • Viscosity due to high-gravity brewing (density of the first wort over 22°Plato), • Viscosity due to the shear forces forming mostly beta-glucan gels or “Oberteig” (upper dough). It has been noticed that differences in temperature during the brewing process have an influence on the mash filterability. This means, that by mashing-in at lower temperatures, we obtain a better mash filterability. When mashing-in at higher temperatures is required, special precautions have to be taken and/or higher malt quality is required. The influence of oxidation, combined with the factors described above, is playing an important role in the mash filterability. The author describes the various tests that have been done: complete oxygen-free mashing-in, mashing-in under normal circumstances (mash inlet from the bottom of the mash tun), differences in brews at different temperatures, and use of enzymes to determine the reasons of high viscosity in the brewing process. The author describes the filtering system used for comparison.

Robert Braekeleirs received a M.Sc. degree in brewing and food technology from the KAHU University in Gent, Belgium, in 1966. He started his professional activity in 1969 at a local brewing company in Gent as production and service manager. From 1972 to 1978, he worked for Interbrew in Leuven as production manager and also for the R&D Department. From 1979 to 1988, he worked for Alfa Laval Brewing Department in Germany, Belgium, and partly for Sweden as a sales manager. In 1989, he joined the Meura Company as R&D manager and became sales & marketing manager in 1997. He has already given different papers and lectures at IGB conventions, VLB Berlin, and other brewing events in Belgium. He is married and has two children.

O-95

Advantages of Fine Wet Milling with a Rotor/Stator System (RSS) and Lautering with a Thin-Bed Chamber Mash Filter (TCM)

DR. HANS-JÖRG MENGER

ZIEMANN Ludwigsburg GmbH

Technical and technological complexity and interdependence between the brewing process steps (brewing) milling, mashing, and lautering gives the targets for the development of wet milling with rotor/stator systems (RSS) in combination with thin-bed chamber mash filters (TCM). Targets of the milling process (milling) are free starch particles that are out of the cellular structure and high permeability of the endosperm cell walls to get optimum surface conditions for native and/or technical enzymes during the mashing process (mashing). The reached husk structure is responsible for an effective and fast mash filtration during the lautering process (lautering). The described points depend mainly on the particle size distribution (PSD) of the mash. The new development RSS allows a calculation of the PSD by using different mathematical and physical basics. Most important for the mechanical effect of RSS is the shear frequency (SF) and the rate of shear forces (VS). Both values depend on construction details of the RSS, which are circumferential speed (VU), outer diameter of the rotor (DR), rotational speed of the rotor (N), and the number of teeth of the RSS. The experience with different sizes of the RSS shows a highly effective mechanical breakdown of the grains and also of the cell walls. This mechanical effect gives a very homogenous PSD that increases the permeability of endosperm cell walls and also the active surface of substrates, which improves amylolysis, proteolysis, and cytolysis. Milling under water protects the mash against additional oxygen pickup, which means less lipoxygenase (LOX) activity, which gives quality advantages. Technical advantages of RSS can be described as fully automatic system and full integration in the brewhouse cleaning-in-place (CIP) system. Positioning is within the brewhouse beneath the vessels without an additional milling building. The RSS is a very compact

construction, which is mill and mash pump in one unit. In case of milling under water, there are no additional explosion and noise emission requirements. After milling and mashing, the lautering step is also important for efficiency and quality. The special design of the TCM chamber plates and the new process technology allow a very homogeneous filling of all chambers within the mash filter. Mathematical and physical basics for the development of the TCM are the filtration laws for porosity cakes, the laws from Darcy and Hagen Poiseuille, and several test simulations in an 8-hL plant. A homogenous filling and a thin-bed spent grains cake are the guarantee for high brew cycles, up to 16 brews per day by high efficiency and best wort quality (low solids/turbidity). Technical advantages are a total automatic system, inclusive of an automatic cloth cleaning device and a low operational/maintenance cost.

Hans-Jörg Menger received the doctor's title for natural science in April 2003 from the University of Stuttgart-Hohenheim, Germany. He began an apprenticeship as brewer and maltster in 1980. In 1985, he started to study food technology at the University of Stuttgart-Hohenheim, Germany. He began employment with Ziemann Ludwigsburg GmbH, Germany, in January 1998 in the technology department. Since April 2000, he is responsible for the patent resort and, since July 2003, he is head of technology department from Ziemann Ludwigsburg, Germany.

O-96

Formation Pathways of Trioxilins During Mashing

LEIF-A. GARBE, Holger Huebke, and Roland Tressl

Technical University of Berlin, Institute for Biotechnology

In contrast to soybean and tomato lipoxygenases, the lipoxygenases from barley and malt (LOX-1 and LOX-2) catalyze the introduction of oxygen into linoleic acid with low regioselectivity. LOX-1 catalyzes the formation of 9-hydroperoxy-10E,12Z-octadecadienoic acid (9-HPODE) and 13-hydroperoxy-9Z,11E-octadecadienoic acid (13-HPODE) in a ratio of 80:20 (9-:13-HPODE) and LOX-2 in a ratio of 31:69 (9-:13-HPODE), respectively. Beside free linoleic and linolenic acids, these enzymes also accept glycerol esterified polar and nonpolar unsaturated fatty acids as substrates. The reactive hydroperoxides are, for example, readily reduced to hydroxides (HODE). In malt 10 ppm free HODE, 109 ppm triacylglycerol esterified HODE and 65 ppm polar esterified HODE were analyzed using isotopic dilution assays (O-18 13-HODE). Further products of the hydroperoxides (HPODEs) are allene oxides that are converted into alpha-ketols, or HPODEs are rearranged to epoxyols that are hydrolyzed to trihydroxyoctadecenoic acids (THOE). The THOE isomers were investigated in detail. The positional regioisomers of THOE are 9,10,13-THOE and 9,12,13-THOE with eight stereoisomers, respectively. Thus, at least 16 isomers of THOE are obtainable and they were assigned in malt by chemo-enzymatic synthesis of eight THOE enantiomers and GC-MS analysis. In malt, various diastereomeric THOE isomers were identified by GC-MS but (9S,12S,13R)- and (9S,12R,13S)-THOE were detected with the highest concentrations. During mashing, a hitherto unknown LOX pathway is activated and only one THOE isomer (9S,12S,13S-THOE) is formed and can be analyzed as free acid in wort and, finally, in beer. This result may indicate a plant defense mechanism throughout the mash process. In contrast to malt lipoxygenases, the new enzyme cascade lipoxygenase-isomerase-hydrolase active during mashing and leading to 9S,12S,13S-THOE is highly regio- and stereoselective and may serve as a plant-signaling compound. The 9S,12S,13S-THOE isomer was formerly described as fungicide in rice blast disease and recently as an antiviral compound. Compared with mono- and dihydroxy fatty acids, the THOE isomers are poorly degraded by yeast and accumulate in beer.

Leif-Alexander Garbe finished his studies of organic and analytical chemistry in 1996 at the Technical University of Berlin (TUB), Germany, with a diploma in chemistry. Afterwards, he worked as an analytical chemist at the Research and Teaching Institute for Brewery in Berlin (VLB). From 1997 to 2002, he was working on his Ph. D. thesis entitled "Metabolic pathways of mono- and dihydroxyfatty acids in yeast" (written in German) and received his Ph. D. degree (Dr. rer. nat.) in April 2002. He performed his Ph.D. thesis at the Department of Biotechnology, Chemical, and Technical Analysis under the supervision of Prof. R. Tressl. During that period, his work as a scientific assistant included the supervision of undergraduate and graduate students of biotechnology and brewery. In 2002, he established a new research group at the TUB focusing on "Microbial-, enzymatic- and chemical formation and cleavage reactions of C-C, C-N and C-O bonds". In cooperation with the VLB, he

performs new techniques, e.g., LC-MS, to analyze trace compounds especially in malt, wort, and beer by isotopic dilution methods.

O-97

Rheological Studies Simulating the Brewery Mashing Process

DECLAN L. GOODE (1,2), Lisa Rapp (1,3), Tilmann J. Schober (1,2), Helge M. Ulmer (1,2), and Elke K. Arendt (1)

(1) Department of Food and Nutritional Sciences, National University of Ireland, University College Cork, Ireland; (2) National Food Biotechnology Centre, National University of Ireland, University College Cork, Ireland; (3) General Food Technology and Food Microbiology, University of Hohenheim, Germany

The brewery mashing process is an enzymatic/time/temperature-dependent degradation process of viscosity creating macromolecules, such as starch and beta-glucan. The measurement of this degradation is gaining interest as both a quality and process control parameter. The aim of this study was to develop a method using a highly sensitive controlled stress rheometer, which could determine viscosity changes in complex systems, such as the brewery mashing process, that contain both dissolved and suspended materials. A controlled stress rheometer, together with a specially designed star-shaped paddle rotor, which enables mash particles to stay in suspension throughout measurement, was used in all experiments. Studies were conducted to simulate an industrial mashing process, taking into account temperature/time, grist loads, adjunct amounts, and enzyme levels. More fundamental studies using pure barley starch and glucan substrates, together with enzyme additions, were also carried out. Four typical viscosity-causing parameters during mashing were assessed in this study: 1) the effects on mash viscosity when increasing the level of unmalted barley in the mash, 2) the effects of malt amylolytic enzyme levels on mash viscosity when using a pure barley starch substrate (pH 5.8), 3) the effects of pH adjustment when using a pure barley starch substrate (pH 4.8), and 4) the effects of glucanolytic enzymes when using a pure glucan substrate. An increase in barley adjunct levels resulted in an increase in start, peak, and end viscosities, together with peak area. These viscosities could be correlated to the endogenous amylolytic and glucanolytic enzymes of the malt and barley. When using a pure barley starch substrate, with an increase in amylolytic enzymes, a resultant decrease in viscosity was observed. A correlation was found between the peak area, peak viscosity, final viscosity, and amount of enzyme added. Using a calibration curve peak area could be directly related to amylolytic activity. Viscosity trends during mashing were found to be greatly altered by the pH of the buffered starch solution. The viscosity readings due to glucans were found to be much less than that caused by starch. The viscosity levels caused by glucans correlated to the amount of added glucanolytic enzymes. It can be concluded from this study that the method developed is a very useful tool for measuring small viscosity changes during the brewery mashing process. It could be used as a screening tool for unmalted and malted grains together with commercial enzymes with regard to their amylolytic and glucanolytic activities. It could also be useful for selecting mash compositions with regard to malt level, adjunct level, pH, enzyme additions, and liquor-to-grist ratios.

Declan L. Goode received a B.Sc. degree in food technology from The National University of Ireland, Cork, Ireland, in 1998. He received his M.Sc. degree in the area of brewing at the National University of Ireland, Cork, in 2001. The title of his thesis was "Brewing with unmalted sorghum and commercial enzymes". He is currently employed as a senior research scientist at the Research Malting and Brewing Facility of the National University of Ireland, Cork, Ireland, where he takes responsibility for the running of the research brewery. He is also working toward his doctorate degree. His areas of research include enzymes and unmalted cereals. He has previously presented at international conferences and has recently published in the Journal of the Institute of Brewing and the Journal of the ASBC.

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The Impact of the Level and Thermostability of Diastatic Power Enzymes on the Hydrolysis of Malt and/or Rice Starch During Wort Production by a Small-Scale Simulated Mashing Procedure

D. EVAN EVANS (1), Helen Collins (2), Jason Eglinton (2), and Annika Wilhelmson (3)

(1) University of Tasmania, Hobart, Australia; (2) University of Adelaide, Adelaide, Australia; (3) VTT Biotechnology, Espoo, Finland

The conversion of starch into simple sugars that yeast convert into alcohol is arguably the most important process in brewing. Not surprisingly, the quality of barley malt is determined by its extract and the degree of fermentability (apparent attenuation limit, AAL) of that extract. For the commercial malt trading, diastatic power (DP) is often used as an approximation for AAL, since DP is more simply and quantitatively measured, particularly since there is a significant impact of yeast strain/source on alcohol yield. DP is a measure of starch-hydrolyzing enzymes that are the combined activity of beta-amylase, alpha-amylase, limit dextrinase, and alpha-glucosidase. However, the measurement of malt DP does not always accurately predict the level of fermentable sugars generated during mashing or the subsequent fermentability of the resultant wort. We have previously shown that not only the amount of DP activity but also the thermostability of the DP enzymes is critical in determining fermentable sugar yield. This is because the mashing temperature program is a balance between the temperature required for starch gelatinization, to enable efficient hydrolysis, and the rate of thermal inactivation of the DP enzymes. In a recent review of starch in brewing, Bamforth (2003, TQ MBAA 40:89-97) identified that there is a dearth of practical information for brewers on the production of fermentable sugars. In this study, seven commercially sourced malts were used for small-scale simulated mashing trials to investigate the impact of differences in the level and thermostability of malt DP enzymes on the resultant wort fermentability. A modified EBC programmed mashing procedure was employed with mashing-in temperatures ranging between 45 and 76°C. Surprisingly, malt extract yield varied little with mashing temperature for most varieties in this temperature range. However, the fermentability of that extract was considerably affected by mashing temperature, with 65°C achieving the highest fermentability for all malt varieties with or without the addition of rice adjunct. In conventional all-malt mashes, the level of fermentability was determined primarily by beta-amylase thermostability with higher levels of DP, limit dextrinase, and alpha-amylase adjusting AAL higher. Mashes that included 30% gelatinized rice in the grist bill showed a similar pattern, except that the low beta-amylase thermostability type (Sd2L) showed a remarkably higher level of fermentability. It appears that an interaction between the Sd2L beta-amylase and increased limit dextrinase activity is more favorable in fermentability terms than would be expected in rice adjunct mashes. The implications for the selection of malt by brewers to optimally suit different brewing styles and regimes are discussed.

Evan Evans graduated from the University of Melbourne with a B.Agr. Sc. degree (Hons) in 1986. This was followed with a Ph.D. degree in 1990, also at the University of Melbourne, which investigated the merits of pollen selection for oil characteristics in canola. In 1990, he moved to Purdue University (IN, U.S.A.), as a postdoctoral fellow to work on improving soybeans for the production of better-tasting soy milk and tofu by using null variants for lipoxigenase. In 1992, he joined the South Australian Barley Improvement Program, where he developed his interest in malting barley and brewing. Recently, he has relocated to the University of Tasmania, where his brewing research interests continue to be in improving malt quality to improve beer quality and the efficiency of the brewing process. Dr. Evans is currently serving on the IGB Awards Committee and is a member of the editorial board for the Journal of the ASBC.

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Degradation of Beta-Glucan Gel in Model Systems and Unfiltered Beer Due to High Hydrostatic Pressure Treatment

STEFFEN FISCHER, Winfried Russ, and Roland Meyer-Pittroff
Lehrstuhl für Energie und Umwelttechnik der Lebensmittelindustrie

In the beverage industry, high hydrostatic pressure is, up to now, predominantly used for the stabilization of fruit juices or milk products. This sparing method conserves valuable nutritious ingredients and flavor compounds much better than thermal treatment. But as shown in former examinations, the high-pressure treatment is not limited to this application. For example, the filterability of beer increases after high-pressure treatment of at least 300 MPa over 300 s. It is already known that this phenomenon depends on the degradation of beta-glucan gel, which has negative influence on the filterability. Beta-glucan can exist in gel and sol states and in the form of solvated molecules. The sol state and the solvated molecules have no negative influence on the filterability. In this work, the reasons for decreasing the content of beta-glucan gel were investigated. Also, the changes in the state of beta-glucan after high-pressure treatment

were examined. The measurements were carried out in model systems with concentrations of 400 and 800 mg of beta-glucan gel per liter. Both in beer and in the model systems, the decreasing contents of beta-glucan gel could not be detected after high-pressure treatment. To identify, whether beta-glucan gel is degraded into beta-glucan or still exists in sol state, viscosity was measured. The influence of the different concentrations of beta-glucan gel is shown in the higher viscosity of the 800-mg sample. Thermal treatment converts beta-glucan gel into sol state. This was approved by detecting a nearly constant viscosity for the 80°C-temperated sample. After high-pressure treatment at 500 MPa, viscosity was clearly decreased. The evidence for the degradation of beta-glucan gel to beta-glucan was supplied. This result was verified by NMR measurements. To analyze the influence of time during high-pressure treatment, online measurements were carried out. These examinations showed that, already during the pressure increasing process, the content of beta-glucan gel is reduced. This effect stops at 200 MPa. Further degradation does not occur until 300 MPa, after 300 s of treatment time, no further degradation could be determined. At 400 MPa, the content of beta-glucan gel is even lower after 300 s of treatment time. There are two different mechanisms for degradation of beta-glucan gel. The first depends on deforming forces during the pressure increasing, the second on the pressure sensitivity of hydrogenous and electrostatic bonds.

Steffen Fischer received a diploma in brewing technologies from Technische Universität München in Weihenstephan, Bavaria. He began his dissertation in 1998 and became a scientific assistant in 2000. Since 2000, he has been giving exercises and lectures in thermodynamics; boiler, power, and refrigeration plants; and high pressure in the food industry.

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Application of Lactic Acid Bacteria in Malting and Brewing

HELGE M. ULMER (1,2), Almudena Soriano (3), Declan L. Goode (1,2), and Elke K. Arendt (1)

(1) Department of Food and Nutritional Sciences, National University of Ireland, University College Cork, Ireland; (2) National Food Biotechnology Centre, University College Cork, Ireland; (3) Department of Analytical Chemistry and Food Technology, University of Castilla-La Mancha, Ciudad Real, Spain

The malting and brewing industry is facing an ever-increasing challenge to be more cost effective, while at the same time improving product quality and safety. The results of this work present the advantages of using lactic acid bacteria (LAB) as a natural way to improve the quality and processability of malt and beer at lower production costs. LAB isolated from grain, malt, and brewing environments were screened for biopolymer degrading enzymatic activities (protease, glucanase, amylase) as well as for the expression of microbial inhibition. A selected number of strains, which either produced high levels of lactic acid, expressed enzymatic activities, or exhibited antimicrobial actions, were used to biologically acidify barley during the malting procedure. The trials were carried out in a micromalting plant. The biologically acidified malt was compared with both a chemically acidified malt and an unacidified control malt. The resulting malt quality was evaluated using EBC Congress mashing analysis procedures. The addition of LAB starter cultures improved viscosity and filterability, increased TSN values, and reduced the microbial load during germination. The LAB, which expressed enzymatic activities, were used for biological acidification of mash and wort in laboratory-scale systems as well as a 10-hL pilot-scale brewery. Wort and beer analyses were carried out according to EBC standard methods. The influence of the enzymatic activities of the strains was clearly seen in the results. The addition of enzymatically active strains led to improved processability (lautering, attenuation), lower beta-glucan levels, and better foam stability, flavor, and shelf life when compared with the controls. The efficiencies of the strains were then challenged in trials in which various levels of unmalted barley (up to 50%) were used. It was found that the brewing samples with LAB performed better than the control samples. The incorporation of LAB enabled the addition of a significant amount of unmalted barley without compromising on processability and final beer quality. Overall, it can be concluded that the selected LAB in this study can be used as a natural way to produce malt and beer of improved quality, processability, and safety while at the same time reducing the production costs.

Helge M. Ulmer received his Diploma Engineer in brewing and beverage technology from the Technical University of Munich-Weihenstephan, Germany, in 1998. He finished his Dr.-Ing. at the Institute of Technical Microbiology, Technical University of Munich-Weihenstephan, Germany, in 2002. The title of his thesis is "Molecular mechanisms of the high pressure inactivation of beer spoiling Lactobacillus plantarum". He is currently employed as a postdoctoral research scientist at the Research Malting and Brewing Facility of the National University of Ireland, University College Cork, Ireland. His current areas of research include application of starter cultures in malting and brewing, development of alternative functional foods in brewing and baking, and the introduction of immobilized lactic acid bacteria fermentation with additional enzymatic activity into the brewing process.