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WBC 2008 Abstracts

Invited Symposia Presentations

EBC Symposium: Beer and Nutrition

I-1

Do raw materials determine healthy beer?

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There is an ever-increasing trend to link health with lifestyle habits, not least of which is an awareness of how food and drink consumption affects wellbeing. Highly publicized scares have created a strong negative relationship between foods and health. Variant Creutzfeldt-Jakob disease, benzene in Perrier, acrylamide in carbohydrate-containing foods and drinks all help to orient consumers to increasingly evaluate foods and drinks in health terms. Conversely, foods and drinks, where possible, are being marketed in terms of their health-positive attributes. Additionally, perceived social status is considered to be partly defined by consumption habits. Arguably, consumers paid less attention to the negative health implications of foods and drinks in the past, perhaps assuming inherent safety, but today this cannot be considered the case. In this paper, the specific example of beer and its impact on health and wellbeing is considered. The key components of beer will be classified according to whether they are positive or negative for the wellbeing of those consumers who drink "in moderation". (The area of "over consumption" will not be considered in detail, except to indicate the health impacts beyond those of alcohol itself.) A distinction will be made between components that have a detrimental impact on wellbeing, both in terms of general human wellbeing (*eg* nitrosamines, mycotoxins) and certain sector-specific sensitivities (*eg* gluten, sulfite). The implications of possible changes in the impact of beer on consumer wellbeing as beer ages will be discussed in the context of the potential impact on legal enforcement of maximum beer age. This classic reductionist view will be expanded to consider some of the possible interactions between components that affect wellbeing. The strategies that brewers can pursue to reduce the risks associated with health-negative attributes will be highlighted, including supply chain management, clear labeling and proactive precompetitive activities that reduce risk to consumers.

I-2

Beer and health: The latest facts

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A brief historical overview of studies on the benefits of moderate drinking that began in the 1970s with support from ABMRF/The Foundation for Alcohol Research will be provided. The overview will open with our ongoing goal: to influence alcohol research in a shift from a problem-oriented approach to examine how the whole spectrum of use affects health and behavior. The overview will include attention to specific studies that have reported benefits of beer drinking related to risk of coronary heart disease, from Arthur Klatsky and from the Augsberg study in Germany. Moderate consumption of alcohol has more recently been shown to decrease the likelihood of developing type 2 diabetes, a disease which is heavily linked to obesity and is a major risk factor for all vascular diseases. The role of diet will be considered in the development of non-alcoholic fatty liver and compared to liver disease due to excessive consumption of alcohol. New data regarding the relationship between alcohol consumption and thrombotic stroke will also be addressed. ABMRF/The Foundation for Alcohol Research is an independent non-profit institution organized for the support of medical, behavioral and social research on the use of alcohol beverages and the prevention of alcohol related problems. It is supported by the brewing industry in the United States and Canada. This unique relationship is unlike other foundations in the health field because ABMRF/The Foundation for Alcohol Research serves as a bridge between industry and the scientific community. It is a model of cooperation between industry and academia for developing new knowledge about the effects of alcohol beverage consumption, especially light to moderate amounts, on health and behavior.

I-3

ERAB engaging in putting knowledge on alcohol and health a step forward

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The European Research Advisory Board (ERAB) was officially launched in 2003 thanks to the financial support of the brewers of Europe. Large and small European breweries directly allocate each year a half-million euro (US$770,000) to support alcohol research; 90% is distributed to research and 10% to administrative costs. This partnership has succeeded in promoting collaboration between the brewing industry and academia. Furthermore this collaboration has accomplished the preservation of the independence and integrity of ERAB by maintaining a strict separation between the source of support and the decision to finance the most meritorious projects. ERAB supports projects in the fields of genetics, liver, cardiology, epidemiology and neuroscience, as well as in the psychosocial field. The results from research studies granted by ERAB illustrate the profit of such collaboration between industry and academia in enhancing health and quality of life.

I-4

The image of 'wholesomeness' of beer: Challenges and opportunities for marketing

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The concept of wholesomeness has come to beer only recently despite the archaeological and historical evidence to the contrary. Beer must now compete with red wine for the accolade of having positive beneficial effects when consumed in moderation. Beer provides far more opportunities for the use of functional ingredients and other additions than wine. However, sales success depends on a number of factors. The first and most obvious problem is understanding what functionality should be in the beer and what, in some countries, is permitted. The second problem is understanding the motivations of customers to drink and to drink a new functional product with an image of wholesomeness. The standard approach used by companies seeking to design or improve their products or services is conjoint analysis. This is in essence a process in which the detailed descriptors of a product or service are found, and a measured mapping is developed to allow candidate products or services to be evaluated. The problems of conjoint analyses are well known and arise even in well-designed studies. Principal amongst them are respondents developing simplification strategies with complex products distorting results, the difficulty in reducing stated perceptions to the underlying attributes, the difficulty in excluding old brand loyalties and the inability of respondents to express clear views on new categories. This paper describes the conjoint value hierarchy (CVH) approach very briefly and how this approach generates useful information about products and possible marketing approaches while not suffering the disadvantages of traditional conjoint analysis. The paper draws on three cases in the FMCG area. The first case supplies background on trends in retailing, the second uses the CVH to gain a better understanding of the factors that are important to retailers at the practical level. In the third case, the CVH is used to evaluate the motivations to drink and the factors important in the drink. The results of these studies demonstrate that the CVH was capable of discriminating between the key factor in product development and marketing. With reference to beer, the CVH analysis showed that the value attributed to a beer by the customer is a complex mix of factors and includes the social benefits of drinking, the physical benefits of drinking, product quality, properties of the drink, an attractive presentation, a satisfactory buying experience, inducement to drink and the image of the company. It also showed a clear distinction between different demographic groups which would allow marketing teams to target their efforts more effectively.

I-5

Changing perceptions about beer

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Beer consumption in Spain had decreased dramatically since 1992. Beer was perceived to be a predominantly "male" drink that was unhealthy and fattening, and beer consumption was negatively impacted by strict legislation enacted in 1995. In 1998, Cerveceros de España (The Brewers of Spain Association) decided to take action and foster consumption of beer. Cerveceros de España worked to develop an effective communication campaign in order to implement it, targeting consumer audiences in general and the female population in particular, as well as scientific, health and nutrition communities and government legislators. In addition, the opinions of sommeliers, gourmets and people keen on gastronomy and leaders in this topic were considered. The principal objectives were to generate favorable opinions about moderate beer consumption and to position beer as a healthy and natural product, linked to Spanish culture. The strategies were focused to research the healthy properties of beer and associate the product with health by promoting scientific and informative initiatives which would be communicated in the media and, therefore, reach scientists and society at large. A Beer and Health Information Centre and a Technical Advisory Committee were created to study the nutritional, functional and health properties of beer and provide a credible and scientific framework for the information. Several scientific studies were commissioned by independent and prestigious entities; activities in this area are being held each year. A non-conventional advertising campaign was implemented, utilizing radio programs, advertising reports in the press and ad placements on TV. Different opinion surveys were circulated to the media each spring and summer to support the campaign's basic messages. In order to promote only moderate and responsible consumption of beer and to prevent misuse or inappropriate intake, especially by young people, minors and drivers, Cerveceros de España developed several social awareness campaigns: "An Inch of Foam, a Mile of Mind" focused on young people and emphasized the drinking of beer in moderate quantities and the enjoyment of social moments involved with this activity; "The Road Demands You Be Alcohol Free" was emphasized that drivers should not drink alcohol if they are going to drive, recommending they drink alcohol-free beer; and "The Parents Have the Word" was an educational program designed to help parents teach their children to make responsible decisions about alcohol. Cerveceros de España also reinforced their commitment with self-regulation through their Self Regulation Advertising Code, which sets more restrictive rules than the Spanish laws, protecting consumers and minors specially. Furthermore, the sector is carrying out a wide lobbying campaign with national and regional authorities, social organizations and political parties with the purpose of showing the efforts toward social responsibility and stopping more restrictive legislation.

I-6

Beware of the beer belly—The fatality of 'beer and health' campaigns

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Beer and other alcoholic drinks can form a pleasurable aspect of a balanced lifestyle. However, I do not think that brewers should overtly market their products claiming health benefits. After all, beer is not a pharmaceutical. Moreover, these campaigns inadvertently help to drag beer into an arena of public debate over disease, mortality, social control, policing and legality, where brewers are soon out of their depth. Consider this: 1) Japanese eat very little fat and suffer fewer heart attacks than Americans; 2) Mexicans eat a lot of fat and suffer fewer heart attacks than Americans; 3) Africans drink very little red wine and suffer fewer heart attacks than Americans; 4) Italians drink excessive amounts of red wine and suffer fewer heart attacks than Americans; and 5) Germans drink a lot of beer and eat lots of pork sausage and suffer fewer heart attacks than Americans. Conclusion: eat and drink whatever you like. Apparently, it's speaking English that kills you.

IBD Symposium: It's Education, Stupid!

I-7

An overview of the IBD professional qualifications

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The IBD is a global membership organization and educational charity, with a core vision statement of "The advancement of education and professional development in the science and technology of brewing, distilling and related industries". In the manufacturing workplace of the 21st century there are constant pressures on cost and performance, and employers deploy a raft of strategies to ensure that they remain competitive. Such strategies can be generally be grouped under the umbrella of "world-class manufacturing". Successful implementation of such programs is dependant on the implementation of key foundation blocks—with the most critical being a total commitment to learning and knowledge acquisition throughout the organization. In supporting the brewing and distilling industries, the IBD provides a suite of globally recognized qualifications, which offers an entry point for operators and technicians (Certificates in Brewing and Packaging) through to a masters level qualification for senior professionals. This paper will provide details of the format of each examination and how each level can be used to create a "ladder" of learning in the workplace. Each level of examination is supported by a comprehensive syllabus, and for all levels below the Master Brewer qualification, there are also tailored learning materials. The Master Brewer qualification is not supported by learning materials, as the exam format focuses on an assessment of a candidate's practical knowledge and work place experience. The syllabi and examination papers are overseen by the IBD Board of Examiners. The Board meets as required to prepare papers for each examination, to maintain the relevance of the syllabi and to reflect changes in knowledge and technology. Currently the IBD offers the entry point examinations (General Certificates) twice a year and the Diploma and Master Brewer examinations once a year. The IBD administers examinations in over 80 centers around the world to facilitate access for candidates. In 2007 these centers were used by over 1,200 candidates. The IBD supports candidates through various training options. In addition to residential courses, the IBD has also established a network of "approved trainers" who are accredited to teach either entire syllabi or subsidiary modules within each qualification. The paper will provide examples of how this network operates in practice and how it delivers training close to the customer. The IBD also offers distance learning facilities, and the paper will provide details on how these programs operate.

I-8

Asset care: How to maintain your most important equipment—People

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One of the most glibly used phrases in industry for many decades has been "People are our most important assets". This statement is used with wild abandon by senior executives and management to impress and influence investors, unions, and other key stakeholders but is rarely actually felt by the 'important assets' themselves. Indeed I would argue that in most cases the physical assets (plant and equipment) gain far more attention than the human assets, who also require regular maintenance, calibration and upgrade. It is critical, of course, for business to retain and develop their people. Retention is all about salary, benefits, reward and recognition, performance management, etc. But, what is people development all about? Many terms have been used to express the important business initiative of people development, including learning and education, training and development, human resource development, competency development/acquisition, etc., etc., etc. This plethora of terminology reflects not only the ever-changing face of people development but also how it is often both misunderstood and mismanaged. This paper will try and look at what people development is really all about and how it is managed in the workplace. Having spent the last nine years of my life almost exclusively involved in people development, I will examine what I believe are the 10 biggest mistakes gen-

erally made by companies in this respect and, through this, hopefully try and identify how to avoid these pitfalls. Therefore, companies will be able to manage those most 'important assets' more efficiently and effectively and achieve performance where it really matters…on the bottom line.

I-9

The rules of the game

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Today's emphasis on being a cost-effective producer of quality malt beverages implies driving step-change, as well as continuous improvement by key performance indicators (KPIs) across the supply chain. This necessary drive must not be at the exclusion of the development of sound problem-solving and interpretive skills in tomorrow's brewers who ultimately contribute to "right-the-first-time", higher quality and an improved cost base. At an extreme, modern-day brewing operations currently risk gaps in the multi-skill and inter-related skill sets necessary to foster and achieve excellence. As an outcome, brewing personnel insufficiently trained to achieve demanded organic growth might well result. This text examines how changes in the "rules of the game" demand a re-focus on technical education in brewing and how alternative options by educational providers offer solutions with either face-to-face, distance learning or blended learning formats.

BCOJ Symposium: Japanese Advanced Technology

I-10

Newest, breakthrough technologies on malt processing for improvement of beer quality

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It is known that ingredients derived from malt have a large influence on beer quality. In order to improve malt quality, many studies on barley breeding, malting, and so on, have been carried out for many years. Among these various methodologies for the improvement of malt quality, development of technologies for malt processing would be the most effective way from the point of view that the quality of malt could be flexibly, boldly and drastically changed. In malt processing the life cycle of the barley, such as breeding and germination, doesn't have to be taken into consideration. Additionally, we can adjust the quantity to treat for use. Therefore, we have tried to develop technologies for producing processed malt that boldly improve the quality of the malt. In this session, new technologies for malt processing that improve malt quality, including malt fractionation technology and technology for treatment with sub-critical H_2O, will be reviewed. The malt fractionation technology is based on the idea that the malt kernel consists of several parts, and major substances that are contained in each part are different. It becomes possible to manipulate the composition of the malt ingredients and to improve beer taste, for example control of astringency, using our malt fractionation technology. As well as creating various flavors in beer through the arrangement of the constituent of various types of malt ingredients, it is possible to improve beer quality characteristics such as flavor stability. The concept of the technology for treatment with sub-critical H_2O is the generation of preferable ingredients from malt to improve beer quality. Not only an effective use of malt ingredients, but also an enhancement of preferable ingredients by malt processing would be necessary to dramatically improve malt quality. It has been found that malt tissue is hydrolyzed by hydrogen ions derived from the H_2O molecule under high pressure and high temperature without acids or catalysts. Using this treatment, several kinds of flavor and aromatic compounds are efficiently generated in a few minutes. Topics concerning the development of the newest, breakthrough technologies for malt processing and the improvement of beer quality will be discussed.

I-11

Control of flavor production in yeast

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Acetate esters, such as isoamyl acetate and ethyl acetate, are major flavor components. While hydrogen sulfide is an off-flavor with an aroma of rotten eggs, sulfite is an antioxidant and plays an important role in maintenance of freshness. These flavor compounds are produced during fermentation and are the most important determinants of beer quality. Acetate esters are synthesized from acetyl CoA and the corresponding alcohols by alcohol acetyltransferase (AATase), and hydrogen sulfide and sulfite are produced during reductive sulfate assimilation in yeast. To understand and control their production in yeast, we have tried a variety of approaches (genetic, gene expression, metabolite levels, and breeding). First, results of the genetic approach have suggested that the reduction in acetate ester production by aeration or the addition of unsaturated fatty acids is due to a reduction in enzyme synthesis resulting from transcriptional suppression of the *ATF1* genes responsible for most AATase activity. Promoter analysis of the *ATF1* gene identified an 18-bp element containing a binding consensus sequence of transcription factor Rap1p, which is essential for transcription activation and suppression by unsaturated fatty acid. Oxygen inhibited the *ATF1* transcript through the Rox1p-Tup1p-Ssn6p hypoxic repressor complex at the binding consensus sequence of Rox1p. *ATF1* expression is activated by nitrogen sources through a protein kinase, Sch9p. Second, we performed a comprehensive analysis of gene expression and levels of sulfur metabolites containing sulfite and hydrogen sulfide. DNA microarray was used to evaluate gene expression, and concentrations of metabolites were measured using GC, HPLC, and CE-MS (capillary electrophoresis-mass spectrometry). This analysis suggests that *O*-acetylhomoserine (OAH) is the rate-limiting factor for production of both sulfite and hydrogen sulfide in bottom-fermenting yeast. Third, we have developed a high sulfite-producing bottom-fermenting yeast strain by integrated gene expression and metabolite levels analysis. Based on the results obtained from gene expression and metabolite level analysis, we hypothesized that sulfite levels could be increased and sulfide levels decreased if the flux from aspartic acid to OAH and the flux from sulfate to sulfide were increased simultaneously. Appropriate genetic modifications were then introduced into a prototype strain to increase metabolic fluxes from aspartate to OAH and from sulfate to sulfite, resulting in high sulfite and low hydrogen sulfide production. To select spontaneous mutants of a bottom-fermenting yeast strain in high sulfite and low hydrogen sulfide production, a mutant resistant to both methionine and threonine analogs were selected and analyzed for similar metabolic fluxes. One promising mutant produced much higher levels of sulfite than the parent, but parental levels of hydrogen sulfide. Finally, findings from our approaches suggested a model for investigating the mechanisms that control flavor production in yeast.

I-12

Investigation of consumer preferences for beer by combined sensory and instrumental analyses

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Many brands of beer are available on the Japanese market. However, up until several years ago, most top-selling brands belonged to the pilsner category and appeared similar in taste to one another compared with the wide variety of beers produced around the world. Recently, along with normal beers and low-malt beers (*happoshu*), non-malt beer-flavored beverages with a broader range of characteristics have been distributed throughout Japan. This presentation reports on a study that evaluated the preferences of consumers who usually drank one particular brand of non-malt beer-flavored beverage. The consumers were recruited as tasters after screening, and their overall preferences for 16 commercial Japanese beers and beer-flavored beverages were evaluated using a seven-point hedonic scale. Additionally, we obtained data from chemical analysis and quantitative descriptive sensory analysis in order to identify the character of each

sample. Based on a cluster analysis of the preference data for each sample, the consumers were grouped into several different categories, including a class of individuals who preferred the non-malt beer-flavored beverages. We created a preference map for each of the categories by combining the consumer preference data and the sensory quantitative descriptive sensory analysis data. Our results demonstrated that these consumers showed a range of preferences for beers and beer-flavored beverages, even though they all usually drank one particular brand. In addition, we were able to examine the correlations between the components, the sensory attributes, and the consumer preferences.

I-13

New aspect of beer evaluation by *kansei* engineering

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Although Japan consumed over 6 billion liters of beer products in 2007, the consumption has not showed a tendency to rise in recent years. There are many commercial brands of beer among three categories (beer, *happoshu*, and beers of a third category), and many new products have been actively introduced to the market in Japan, as brewers have striven to develop new products to fit customer demand. Therefore, we needed to find solutions for individual customer requirements. "*Kansei*" means human feeling or image in Japanese, and *kansei* engineering is a method for translating subjective impressions into objective criteria. *Kansei* engineering extends over the humanities, social science and natural science. We applied this technique to the evaluation of beer flavor and the development of products based upon consumer feelings. This study was aimed at demonstrating the importance of the swallowing motion and human emotion in terms of beer preference. 1) Swallowing motion—smooth swallowing is a key factor for beer drinkability. Swallowing is a serial movement of the mouth and throat in order to transfer a food or beverage from the oral cavity to the stomach, which is achieved by the coordinative activities of the pharyngeal muscles. Along with this movement, the larynx pumps up and down. In order to analyze the beer drinking motion exactly, we measured the larynx motion via an electromyogram (EMG) of suprahyoid musculature and swallowing sound using a non-invasive biometric system. Analytic results of the parameters, such as the period of larynx heave, correlated with the sensory evaluation, suggesting that our biometric system could objectively evaluate throat sensations during the consumption of beer. 2) Human emotion—certain aromas are known to change one's emotions. Psychological conditions, such as comfort, have been estimated on the basis of fluctuation in brain waves. Spectrum information concerning the frequency fluctuation of alpha-waves is related to the psychologically evaluated values of positive/negative moods. In the meantime, the alleviation of stress and changes in human mood during the smelling of beer aroma were investigated using a measurement system for human brain waves. The aroma of beers, namely that characterized by ester or hop flavors, had a relaxing effect on humans. It was expected that ester and hop flavors in beer would contribute to enhanced feelings of relaxation while drinking beer. *Kansei* engineering is a relatively new and effective set of tools for research to concretely determine the feelings evoked by beer products. In the future, a new approach using *kansei* engineering will be able to eliminate the gap between the actual feeling of the consumer, the target feeling, and the ambiguity of feeling caused by the individual character of the consumer.

Oral Presentations

O-1

A flush a day keeps the bugs away

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The understanding of the necessity of cleaning but lack of practice is a quite common game in selling draft beer. Without any doubt, hygiene is a crucial parameter for sales success in the draft beer scene. But the obvious question is, how much care is essentially needed and furthermore which care is leading to a sufficient level of quality? Instead of an unverified assumption, scientific investigations are needed to answer these questions. If we take a closer look at the cleaning process for draft beer equipment there are three major areas—the beer line, the coupler and the tap. In addition we find some country-specific auxiliaries such as fob stops, pumps etc. This presentation discusses the impact of tap cleaning on the microbiological situation for the tap itself and for the whole dispensing system. In other words—is tap care worth the effort? Practical investigations have been carried out with the following experimental design: A test rig consisting of five beer lines identical in length, diameter, construction and mountings was used. All the beer lines were equipped with the same tap design. The beer used for the trials was a German lager beer. In cases of tap care a tap-ball was deployed. One series of tests ran for six weeks and was repeated three times to meet statistical requirements. Concerning the tap care five different parameters were determined: Tap 1, treated daily with water; Tap 2, treated daily with disinfectant; Tap 3, treated with disinfectant once a week after line cleaning; Tap 4, no tap treatment after line cleaning. The total equipment is cleaned chemically on a weekly basis. As a control, Tap 5 was used without a regular cleaning interval to demonstrate the prompt contamination found without a regular cleaning cycle. Once a week two samples from each line were taken and tested for microbial load by plate count (cfu). The first sample indicates the microbiological situation at the tap and the following sample represents the beer line behind the tap or rather the situation of the dispensing system. Daily cleaning of the tap with water or disinfectant shows significant reduction of the microbiological load at the tap but most interesting also for the beer line behind the tap. As early as three weeks there are definite variations in the bio film found on the tap wall. In comparison to the standard weekly cleaning procedure with tap treatment the reduction of microbiological load due to daily treatment is dramatic. In summary it has to be concluded that tap care improves considerably the whole microbiological situation of a beer dispensing system and gives all outlets with high quality standard a competitive advantage in selling draft beer.

O-2

Review on recent developments in dispense hygiene

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Brand loyalty is of the utmost importance to the brewer and nowhere more so than with draft dispense. Despite the perception of keg beer as the most stable packaging format, delivering beer of consistent and characteristic quality from the brewery gate, there is no guarantee that it will reach the consumer's glass unblemished! Inappropriate beer dispense can significantly damage beer quality, leading to a loss of business, not to mention the brewer's reputation. Perhaps the greatest risk to dispense quality is the use of microbiologically contaminated dispense equipment. The early 90s established the foundations of much of our current understanding of microbiological colonization of surfaces, particularly in the brewery but also within the dispense system, and in particular our understanding of biofilm development. This begins when a conditioning layer, consisting of organic material from the beer, forms on the line surface. Bacteria settle on the conditioning layer and produce extracellular polysaccharides which form a highly protective 'slime' coating. Beer spoilage bacteria and yeast are now also able to attach to this sticky surface. The physical 'shedding' of particles from the mature biofilm then ensures its further spread throughout the dispense system. The worst location in terms of biofilm formation and dispense system contamination is usually identified at the dispense tap, where environmental conditions are more favorable for a wider range of microbiological contaminants. BRI and others have recovered a diverse number of viable, non-brewing related microorganisms, not usually capable of survival in beer, directly from tap spouts. This raises particular concerns at sites where beer and food are served in close proximity and in the UK has led to the publication of technical guidelines from the British Beer and Pub Association recommending that, in order to limit the potential for cross contamination, appropriate food hygiene practices should be closely followed in outlets serving both beer and food. To limit the risk of beer quality deteriorating during dispense, frequent line cleaning is recommended. However, no agreed standards exist as to how often cleaning should be carried out. As it is a time-consuming job and beer losses are associated, there is a tendency to extend periods between clean-

ing cycles, potentially negatively impacting the product. A number of new cleaning solutions have emerged on the market over the last few years. These consist of novel, physical processes such as 'slush ice' cleaning, electromagnetic devices and automated line cleaning systems and new chemical approaches such as activated 'water'. BRI has a simulated cellar/bar dispense system where we have been involved in independently testing new developments for their effectiveness.

O-3

Are plastic bottles ready to replace glass as a beer packaging?

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One of the main aspects that influences beer flavor stability is the impact of oxygen. Packaging materials like glass or metal seem to be nearly inert against the permeation of oxygen. Alongside the well-known advantages of PET there is the problem of plastic's inherent permeability to gases (focusing on O_2 and CO_2) that occurs along the partial pressure gradient between the inside to the outside of the package. To enhance the barrier properties of different plastic materials (PET, PEN, PLA, etc.) different bottle systems (multilayer techniques, internal and external coatings, blendings) were developed and improved. The quality differences of these various systems were evaluated through the measurement of permeation. A new test method to measure the permeation through plastic materials will be presented. The real time aging and oxygen-free bottling used for this method imitates the filling and aging process in praxis as closely as possible. The lecture contains a comparison and evaluation of the latest development in barrier enhanced plastic bottles and closures. It additionally gives an overview of how far the industry has come today concerning the possibility of filling quality-brands in plastic bottles or plastic event packs of different plastic materials without losing the flavor stability of the product.

O-4

A novel method for interlaboratory analysis of total package oxygen

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The measurement of total package oxygen (TPO) is a standard procedure in bottling. It provides fundamental information for the prediction and improvement of beer flavor stability. Today's analytical standard procedure has been developed by researchers from Cervecería Polar almost 25 years ago (1). Sufficient repeatability and reproducibility are vital for any kind of measurement. In the case of TPO, repeatability between different laboratories remains an unsolved problem and poses a serious threat to the significance of the analysis (2), especially in large breweries with multi-plant operations. In this work, several different approaches for the preparation of TPO samples were compared, among them modifications of containers and different matrices. As far as the containers are concerned, trials were focused on bottles with pry-off crown corks. A selection of bottles according to their volumetric capacity was necessary in order to minimize errors. The central issue to be addressed was the method of introduction of well-defined amounts of oxygen into the container. Best results were obtained by the use of crown corks custom-fitted with a septum from gas chromatography, allowing the injection of the desired amount of air with a gas-tight precision syringe. Matrix effects were evaluated by performing reproducibility and repeatability analyses with different liquids in the sample containers. In the case of water, several modes of preparation were examined, namely degassed and carbonated water, water with stoichiometric addition of antioxidant to eliminate dissolved oxygen, as well as demineralized or distilled water. None of the preparations yielded a satisfactory reproducibility of TPO measurements. Using beer as a matrix, statistical distribution was significantly better. Preparations assessed included fresh tunnel-pasteurized beer, pasteurized beer aged at 28 or 60°C, and pasteurized beer from the brewery's pilot plant. Combining the most favorable conditions, an acceptable reproducibility and repeatability of 22% was achieved. Based on these findings an interlaboratory analysis model was designed which consists of two different levels of air injection into the beer, thus generating three concentrations of oxygen. This model has been put into practical use at Cervecería Polar and its reproducibility and repeatability is at 24%, thus within the category of "acceptable". This compares to an "unacceptable" 48% calculated from the data presented by the ASBC subcommittee (2). References: 1) Vilachá, C. and Uhlig, K., Brauwelt 124, 754-758, 1984; 2) ASBC, Report of the Subcommittee on Method for Reference Standard for Total Package Oxygen, J. ASBC 65, 238-240, 2007.

O-5

Development of an analysis workflow system at the brewing research laboratory

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In Japan, consumers have taken a growing interest in, and demanded, food safety, quality management and traceability in the food manufacturing process. Accordingly, the quality of associated analysis data has also become an important issue. To ensure high-quality product development as related to beer and related technologies, breweries must have access to highly reliable and timely analytical data. We developed an analysis workflow system to allow Kirin the ability to manage and utilize the large volume of analytical data related to beer, happo-shu, and new genre development. The development process consisted of designing the work flow, implementing the core database system, developing an analysis request system, creating an interface with the pilot plant system, and networking with the analytical equipment. Having developed this system, we have been able to improve analytical work efficiency, and provide new levels of convenience when extracting/utilizing data.

O-6

Environmental considerations and innovations used in the design of a new packaging facility

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A new bottling hall was necessary to support the capacity needs of New Belgium Brewery. To meet this need and to stay consistent with the values and culture established by the organization, a commitment to innovative technology and environmentally friendly engineering was an essential design criterion. For the building design, a number of energy saving design features were included, which significantly reduced the amount of energy consumed by the heating, cooling, ventilating and lighting systems. An energy model was developed to evaluate alternative building systems and used to compare the energy performance against a baseline measurement established by ASHRAE 90.1 2004. The model calculated a savings of 24% greater than this benchmark. Overall, the lighting and HVAC energy usage was reduced by 50% compared to the benchmark. Key elements of the design included a displacement ventilation system which allows for higher supply air temperature than mixed systems. Cooling is provided by a direct/indirect evaporative cooling system. Variable speed drives were installed on all motors. Skylights and day lighting controls, along with efficient lighting systems, were installed. The exterior walls were constructed with 10 inch SIP panels (R-38). Additionally, extensive use of recycled materials was included throughout the building finishes including flooring, countertops, doors and ceilings. The facility also installed the rapid charge system for forklift batteries, eliminating the need for a charging room and associated environmental and personnel hazards. The bottling line had several separate features, including the latest electronic filler, a first in the US. The filler provides quick changeover, and reduced CO_2 consumption, and lower oxygen pickup. Special technology was included to reduce overall water consumption by having a reuse system from the rinser supply the vacuum pump and external bottle wash. New technology was also added to the dry end packaging with the addition of a wraparound packer with a partition inserter for 12 and 24 loose count packages. Finally, at the end of the packaging line the inclusion of the latest in robotic technology was used in the new palletizer. This technology eliminated many mechanical methods for creating case patterns in building the finished pallet.

O-7

The establishment of new yeast management system in our breweries

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It is essential to use vital yeast for making beer of high quality. We have already optimized the time to crop and the conditions to store the yeast, and now we have a process that can maintain highly vital yeast through serial repitching. However, recent studies in our laboratory have revealed that lager brewing yeast suffers from unwanted changes in its chromosomes under oxygenated conditions (1). In practical brewing, such chromosomal changes in yeast may cause aberrant fermentation and deteriorated beer quality. Therefore, it is desirable to establish a system that can detect yeast alteration and predict problems in beer production. We developed a DNA microarray which contained all genes and intergenic sequences of lager brewing yeast (2), and it was confirmed that it could detect changes in genetically altered yeast strains (1). Using this technology we investigated whether some brewing associated stresses (e.g. oxygenation or high temperature) could lead to alteration of chromosomal structure. We exposed a lager yeast strain from our laboratory stock to those possible stresses, and isolated several altered strains. We found that these strains had the deletions and/or duplications of large chromosomes compared with the parental strain. Some of the obtained yeast isolates produced much higher amounts of VDK than their original stock cultures during the fermentation trial. It was very likely that some particular regions could change more preferentially under these stresses. The PCR primers were designed to detect this type of change, so that the test is easily practiced in our brewery laboratories. Furthermore we established a very simple plate assay system that can detect various mutations, and introduced it to our brewing plants. Using these systems our yeast has been periodically inspected by the quality assurance division in our breweries. Yeast management in our breweries has greatly improved with these detection techniques. References: 1) Hatanaka, H *et al.,* Proceedings of the 31st EBC Congress, 2007 : 397-405; 2) Nakao, Y *et al*., Yeast, 2005, 22 (S1), S43.

O-8

Commissioning and start-up of a highly automated 7mm bbl brewhouse in the first new Coors brewery in the United States in 133 years

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'Project Jefferson' was the construction, commissioning and start-up of the first new Coors' brewery in 133 years. This was a green field site installation of a 7MM bbl brewery with twin 1000bbl brewstreams, a high level of process automation and cutting-edge brewhouse technology. An overview of the brewhouse design, automation, systems integration and operational philosophy are reported. The construction, commissioning and start-up of the brewhouse are discussed in detail. Performance acceptance criteria and actual performance of the brewhouse are reviewed with particular emphasis on low shear, low DO pick-up mashing, efficient short cycle time lautering and high evaporation rate boiling.

O-9

An extreme view or just plain old fashioned beer making? How beer proteins suppress beer staling

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'Ultra-stable' beers in our experience contain proteins (Pr-SH) that react strongly with thiol-specific reagents. This means the proteins are in a highly reduced redox state (ratio of thiols:disulfides is high). Beer proteins have peroxidase activity which seems similar to the thiol-dependent peroxidase activity operating in serum albumin proteins. A single thiol reacts with H_2O_2, producing water and converting the thiol to a sulfenic acid residue (Pr-SOH). This oxidized residue is extremely reactive. Nevertheless provided it occurs in a hydrophobic pocket, it is possible for the sulfenic acid to be reduced back to the Pr-SH form. An oxidizable substrate, like sulfite or a thiol compound RSH or even a polyphenol(s) is required. The reaction pathway has been inferred from inhibitor studies with dimedone a compound which reacts exclusively with sulfenic acid residues, and by Western analysis of beer proteins following accelerated tests. These Westerns are constructed to react exclusively with sulfenic acid residues. This allows the cycling between thiols and sulfenic acid moieties to be visualized. After extended periods of ageing this occurs at a slow, albeit significant, rate. Proteins in ultra-stable beers stay highly reduced for long periods, well after SO_2 is exhausted (high level of reactive thiol). We have considered whether peroxidase, superoxide dismutase (SOD), or catalase will thus substitute for free SO_2 under these conditions and control reactive oxygen species. Peroxidase requires an oxidizable substrate, but converts peroxide solely to water. Catalase destroys peroxide but, in addition to water, oxygen reappears. SOD and catalase usually act in tandem; converting the oxyanion to water and oxygen. Peroxidase on the other hand can eliminate oxygen from packaged beverages. In addition to these possibilities we have considered whether small molecules with enzyme-like activity, akin to those above are involved in the suppression of staling in these beers. The high molecular weight fractions prepared from beers by ultrafiltration contain anti-ROS activity akin to conventional catalase, SOD and peroxidase. Similarly low molecular fractions contain catalase and SOD activity, which is heat insensitive, and is not associated with proteins. Although there seem to be synergistic effects when high molecular weight and low molecular weight fractions from stable beers are combined. Both catalase and SOD activity have been detected in plant extracts, notably Rosemary extracts which are not protein based, as well as extracts from hop varieties. We present a model in which proteins and mimetic compounds combine to suppress ROS levels in certain beers. In the long term we think it is preferable to retain functional protein and to extend the donor substrates for peroxidase. We think that having sustaining levels of oxidative substrates in beer, and retaining protein peroxidase activity while in addition maintaining mimetic activities, can be achieved within classic beer-making practice.

O-10

Recent developments in protein-polyphenol haze

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Considerable new information about protein-polyphenol haze has emerged over the last 25 years. Only proteins that contain a significant amount of proline bind polyphenols and form haze. Such proteins have finite numbers of sites where polyphenols can attach. Polyphenols that bind to proteins have at least two hydroxyl groups on a single aromatic ring. Vicinal triphenols bind proteins more strongly than vicinal diphenols, which bind more strongly than m-diphenols. Haze-active (HA) polyphenols have at least two such rings and can bridge proteins together. 'Single ended' polyphenols have only a single binding ring; they can compete with HA polyphenols for binding sites in proteins. Structurally larger polyphenols of the same type are more HA. Protein-polyphenol interaction involves hydrogen bonding and hydrophobic bonding, but not ionic bonding. The proportion of protein to polyphenol affects both the amount of haze (light scattering) and the size of the haze particles. A conceptual model that accounts for this behavior was developed. Weight ratios of protein to polyphenol near 2:1 and 5:1 correspond to larger haze particles and more light scattering than higher or lower ratios. The changes seen as the ratio changes are mainly in the proportions of particles of a few discrete sizes rather than gradual shifts of a monomodal distribution. The pH has a profound effect on protein-polyphenol interaction. Maximum light scattering with the same concentrations of protein and polyphenol occurs near pH 4 and drops off fairly sharply as pH increases or decreases. The pH of this haze peak is far from either the isoelectric point of the protein or the pKa of the polyphenol. Haze formation has two linear phases. At first little or no haze formation is apparent. After some time haze starts to increase linearly. The rate of haze formation appears to be a function of the product of the concentrations of HA protein and dimeric proanthocyanidins. Silica, unlike bentonite, is remarkably specific for haze-active proteins because it binds to the same feature (proline residues) to which polyphenols attach. Silica virtually ignores proteins that are low in proline (including many that are foam-active). Silica is more effective in removing HA protein when the ratio of protein to polyphenol is high. PVPP is more effective in removing

polyphenol when the ratio of protein to polyphenol is low. Binding of HA polyphenol to PVPP and HA protein to silica also involves hydrogen and hydrophobic bonding but not ionic bonding.

O-11

Diagnosis of causes of foam instability in commercial beers

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The cause of foam instability in commercial beers was explored. Three proposed causes of foam instability in beers were investigated: that arising from the presence of lipid, that from lipid hydroperoxides, and that from low molecular weight polypeptides. To diagnose the cause of foam instability commercial beers were ultra-filtered (UF). The unfiltered beer, the permeate and the retentate were then dosed with varying amounts of egg white and improvement in foam stability was observed. Further investigations using immobilized bovine serum albumin (BSA) to adsorb lipid from the original beer and permeate and proteolytic enzymes to hydrolyze any low molecular weight polypeptides passing through to the permeate were used to diagnose foam instability arising from lipid and low molecular weight polypeptides respectively. UF fractions were tested for the presence of lipid hydroproxides. Data was correlated with results from the Steinfurth foam stability tester.

O-12

Investigations on the behavior of organic radicals in barley and malt during the malting and mashing process by electron-spin-resonance spectroscopy

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Barley, malt and spent grain as well as the malting and mashing process were investigated by using Electron-Spin-Resonance (ESR) spectroscopy. For solids a method using a new reference signal was applied. The ESR spectroscopy is a rapid method for detecting ions and organic radicals containing unpaired electrons. It can be used for liquid and solid samples. Besides liquid measurements, ESR-spectroscopy, using Mn^{2+} as an internal standard, has been used in the past to control the concentration of organic radicals in malt or green malt and their development during the malting process. The new reference signal is detectable directly besides that of organic radicals in the ESR spectrum and allows a better quantitative detection of organic radical concentration. Influences on the sample also have an impact on the reference signal, because the substance responsible for signal generation is positioned next to the sample in the spectrometer. Based on this background, it is possible to analyze the radical concentration in barley, malt and spent grain quantitatively. Besides low temperature ESR-measurements (77 K) for the detection of typical metal ions in malt, the new reference signal has been used for the investigation of organic radical concentration in different fractions of malt samples as well as the development of organic radicals during the mashing and malting process under different technological conditions, such as steeping degree, germination time, withering and curing under different atmospheres like oxygen, nitrogen and CO_2. The results show different concentrations dependant on certain malt fractions. The highest concentrations were located in husks, whereas the lowest were found in the endosperm. Therefore a correlation between extract yield and radical concentration in spent grain with respect to mass was achieved. It could be shown that mashing conditions also have an impact on the concentration of stable organic radicals, because they are able to react partially with organic reactants during the process. Based on this background it is possible to use this method to investigate spent grain analysis via ESR to observe influences on mashing, e.g. the applied mashing process (temperature, time, rests) or the use of sonic waves. Withering and kilning have a major influence on radical generation in malt depending on malting conditions. A strong increase in radical concentration during withering and kilning shows high stress conditions and intensive oxidation reactions. Investigations on barley have shown that the concentration of organic radicals can vary strongly depending on environmental and storage conditions (humidity, temperature, drying).

O-13

Hops and health

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Hops has been declared the "German Medicinal Plant of the Year 2007". This award makes it obvious that hops is not just a raw material for beer production but is also a recognized natural medicinal product. Mankind presumably used hops as a healing plant for a long time before discovering during the Middle Ages that it has preservation properties as a beer ingredient. Since the early Middle Ages, use of hops in folk medicine has been documented. It served as a remedy for treating a variety of diseases, e.g. stomach complaints, ear infections or toothache. It is noteworthy that such uses of hops were known both in European and in Indian-Ayurvedic medicine and were also common in a large number of Native American tribes in North America at a time when no transfer of knowledge could have taken place between continents. Hops continued to be used for healing purposes for centuries. Today, it is mainly recommended for calming and to aid sleep. The effectiveness of hops is attributed to its many positive constituents, above all bitter substances and polyphenols. Especially in the last decade, pharmacological research into the positive health aspects of hops and hop constituents has been considerably stepped up. Accordingly, scientific investigations are ongoing worldwide. They are providing new information continuously that contributes to an understanding of medicinal properties that have been used for centuries. In the case of α-acids and β-acids, the best known hop bitter substances, manifold antibacterial, but also anti-inflammatory and even carcinostatic, properties have been discovered. Mixtures of certain hop polyphenols have proved to be effective against bacteria that cause caries or allergies. The prenylflavonoids in hops seem to be particularly interesting. They are also classed as polyphenols but occur relatively rarely in the plant world. The best known is the prenylflavonoid xanthohumol, mainly because of its cancer preventive potential, which is of exceptional interest. Some of these hop constituents with proven positive effects are carried over unchanged into beer during brewing (e.g. rutin, the glycoside of the potent anti-oxidant quercetin); others are converted chemically (e.g. isomerizations of xanthohumol to isoxanthohumol or α-acids to iso-α-acids). A multitude of positive effects has also been shown to be associated with these isomerized compounds, e.g. isoxanthohumol is effective against osteoporosis and iso-α-acids against diabetes. All these nutritionally valuable beer ingredients originating from hops as a brewing raw material promote the image of beer and provide some convincing arguments in the discussion about beer and health.

O-14

About celiac disease and beer – 2

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Celiac disease is a reaction of the gut lining to small peptides in the diet formed during digestion of cereal gluten molecules, especially those of wheat. Barley also contains gluten, though much less than wheat, and because of significant modification in malting, and dilution with water and adjuncts during brewing, barley proteins only minimally survive into beer; as a result most beers test negative in rudimentary ELISA methods for detecting gluten fragments. Nevertheless most celiac patients follow their physician's advice and eschew drinking beer, because the potential negative impact of ingesting gluten can be severe. For this reason there is currently some interest in making gluten-free beers from approved non-gluten-containing materials such as sorghum. While there are many alternative beverages for beer drinkers to enjoy, there is really no alternative for wheat-based foods and so physicians are exploring an enzyme that, taken along with such foods, prevents the formation of the gut-damaging gluten fragments. Given that brewers are well versed in managing cereal proteins and enzymes there seems no reason why this enzyme should not be tried in beer-making processes.

O-15

The origin and transfer of silicon in beer

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Recently, silicon has been shown to be a healthy component of beer. The silicon content of beer was investigated by analyzing brewing ingredients

and samples throughout the brewing process using nitric acid/hydrogen peroxide/hydrofluoric acid microwave digestion and analysis by Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES). Several popular brewing techniques were performed to determine what effects these processes have on the extraction of silicon into the resulting beer. Commercial packaged beer samples were also analyzed with regard to beer style and origin to determine the silicon content.

O-16

Effects on the formation of crystalline silica phases during fluxcalcination of kieselguhr

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Kieselguhr is mainly composed of the skeletons of diatoms, a unicellular aquatic plant related to algae. These skeletons consist of opal-like, amorphous silica, containing small amounts of microcrystalline structures. Kieselguhr is used worldwide for deep bed filtration processes in the food and beverage industry. Due to its excellent characteristics as high absorptive capacity, surface area and chemical stability, alternative filter aids could not prevail. Prior to using kieselguhr as a filter aid, the raw material must undergo a conditioning process. Three types of kieselguhr have to be differentiated: dried, calcined, and flux-calcined. Flux-calcined kieselguhr is manufactured by treating the kieselguhr in a kiln at 1620°F–2200°F after adding an alkaline flux, generally sodium carbonate. Porosity and specific surface area strongly decrease and most of the amorphous SiO_2 is transformed into a crystalline phase of SiO_2 called cristobalite. Crystalline phases of SiO_2 can cause silicosis and are suspected to cause cancer. Therefore the influence of fluxing agents on the formation of cristobalite during the fluxcalcination of kieselguhr was investigated. Dried kieselguhr was treated at temperatures between 1620°F and 2010°F after adding either Na_2CO_3 or K_2CO_3 as a fluxing agent in concentrations from 1.0 to 6.0 mass.-%. The duration of the heat treatment varied from 1 h to 4 h. Amorphous and crystalline SiO_2 phases were differentiated via X-ray diffractometry (XRD). Samples containing sodium carbonate powder as fluxing agent showed the tendency to form crystalline structures. Samples containing potassium ions showed a significantly lower tendency to form crystalline structures. Adding the fluxing agent as aqueous solution advanced this effect due to the optimal distribution of potassium. Samples were flux-calcined in melting pots in a ceramic oven. Furthermore the Seitz water value of the samples increased in contrast to the raw material. Using Na_2CO_3 the water value of the flux-calcined samples was greater than using K_2CO_3 as a fluxing agent. In contrast to the white color of the samples treated with Na_2CO_3 the color of the samples treated with K_2CO_3 was brown. At the moment the influence of other fluxing agents such as NaCl, KCl, $CaCO_3$, $CaCl_2$, CaO, $MgCl_2$ and MgO is examined in order to increase the water value and the degree of whiteness of flux-calcined kieselguhr. Furthermore kieselguhr will be fluxcalcined in a rotary kiln and in a circulating fluidized bed reactor, in order to investigate the influence of the kiln design on the formation of crystalline silica. Results will be given in the presentation at WBC.

O-17

Re-inventing depth filtration—Purifying the brewing process

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Factors within the brewing process that can affect the quality of the beer have been present since the first "mead" was made around 1700 BC and the first depth filter was engineered. For decades, minerals found in depth filters were unavoidable. Using depth filtration, brewers have had to contend with and control issues affecting the brewing process such as mold, introduction of heavy metals like iron, and inorganic materials, all of which can affect beer flavors. By eliminating these factors one can "purify" the brewing process, reduce the cost of filtration, participate in "being Green" and ultimately produce a better beer. We will show the effect of heavy metals in the brewing process and what effects they have on making beer. Due to new technology we will demonstrate how eliminating minerals can make the brewer's job easier. And, we will show results from industrial scale testing to prove that by re-designing depth filtration the brewer can take a huge step in purifying the brewing process.

O-18

Cross-flow membrane filtration at the Coors Shenandoah brewery

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Coors Shenandoah installed the largest 'PROFi' centrifuge + membrane filtration system in the world in 2007. The capacity of the green field site brewery is 7 MM bbl. This system was installed to continuously filter all high gravity beer for packaging. Decision criteria for selection of cross-flow filtration in preference to kieselguhr systems are reviewed. The system was designed based on successful pilot filtration at the Coors Golden brewery. System design and control are reported. Experiences with the PROFi system during commissioning, start-up and full production are reported. Performance claims about minimal dissolved oxygen pick up, minimal beer losses during operation and changeover, low temperature pick up during centrifugation and filtration and excellent beer clarity were all substantiated during operation. Critical factors for efficient continuous filtration were found to be throughput per filter block, centrifuge cleaning frequency, filter block cleaning duration, beer temperature, outlet turbidity from the centrifuge and cleaning chemical concentration, temperature and pressure.

O-19

Improvement of beer flavor stability by the reduction of the protease activity in malt

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Compared to other alcoholic beverages, beer shows poor flavor stability. Therefore many studies have been carried out to improve beer flavor stability, in particular on the suppression of trans-2-nonenal (T2N), which presents a cardboard-like flavor. In the ASBC 2006, we also have reported a method that suppresses the formation of T2N by controlling lipoxygenase (LOX) activity of malt to improve beer flavor stability. However, not only the cardboard-like aroma may be responsible for the stale flavor of beer, in particular in all-malt beer. Because other characters, expressed as "soy sauce-like", "honey-like", and "cooked potato", raise the score for stale notes additionally, we cannot suppress the stale flavor of beer enough only by controlling cardboard-like flavor. Two pathways are most often accepted as the major contributors to beer staling: lipid oxidation and amino-carbonyl reaction. By sensory evaluation using a spiked sample with methional, we confirmed that methional seemed to be the compound related to soy sauce-like flavor. Moreover, addition of methionine to wort in brewing process led to a higher level of methional in the aged beer. So, focusing on the formation of Strecker aldehydes derived from the amino-carbonyl reaction, we have tried to control the protease activity of malt in order to reduce the amino acids content of wort. Because the thermal stability of protease is comparatively low like LOX, we have treated malt with a pressure cooker for a short time. Brewing trials in the laboratory with the treated malt demonstrated the reduction of stale flavors like soy sauce in forced aged beer, and the improvement of beer flavor stability could be achieved. It was observed that the content of specific amino acids such as methionine in wort was decreased by low protease activity and finally the formation of methional decreased in aged beer. From these observations, we found that we could improve beer flavor stability by depressing the protease activity of malt, because soy sauce-like flavor in stale beer is partly caused by methional derived from methionine in wort.

O-20

Changes in protein and amino acid composition during malting—A comparison of barley and oats

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Barley (*Hordeum vulgare*) has been traditionally used for the production of malt, whereas malted oat *(Avena sativa)* was widely used in medieval times and before. Nowadays oat malt is used in the brewing industry as a

flavor adjunct for the production of special beers. The most important aim during malting is to generate starch degrading enzymes. However, the protein content of malt is of central importance with regard to filtering, fermentability, foam and haze stability. The purpose of this study was to evaluate the changes in protein and amino acid composition from the raw barley and oat over germination to the final malt using a range of methods. After extracting the cereal proteins on the basis of their solubility (Osborne fractionation) the different protein fractions were analyzed using a Lab-on-a-Chip technique, which separates the proteins, based on their molecular weight, by capillary electrophoresis. This new technique for the analysis of proteins was supported using two-dimensional gel electrophoresis. In addition, amino acid analysis was carried out, using a chromatographic method. The proteolytic activities of the grains were measured at various stages during malting. It was found that the overall proteolytic activity increased during germination. The values reached for barley after malting (21.39 mg g^{-1} h^{-1}) were slightly higher than those of oats (20.31 mg g^{-1} h^{-1}).Results of the Lab-on-a-Chip analysis revealed that protein degradation during malting was higher in barley than in oats. Especially the storage protein fraction of barley (hordeins), as well as the glutelin fraction, was degraded completely, whereas the oats prolamin and glutelin fraction were not entirely degraded. In the main protein fraction of oats (globulins) many proteins could be detected, where only a few were found in the barley globulin fraction. In both albumin fractions, which contain the metabolically active proteins, increases of proteins could be observed. The results obtained from the two-dimensional gel electrophoresis followed the same trend as the Lab-on-a-Chip results. A deeper understanding of the protein changes was achieved by amino acid analysis of the unmalted and malted grains. Due to the higher protein content of oats, detectable amino acid levels were higher in oats than barley. Glutamic acid was found to be the amino acid with by far the highest concentration of all amino acids in both barley and oat grains. Amino acid composition of the grains was comparable.

O-21

Heat treatment of barley restricts *Fusarium* activity during malting

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Several filamentous fungi, including fusaria, are sensitive to heat. In this study barley was heat-treated prior to the malting process in order to inactivate the *Fusarium* fungi during malting. Two-row Scarlett and six-row Tradition barley samples were exposed to heat (60–100°C) for 5–10 seconds prior to steeping. In addition to traditional culturing techniques, the changes in the *Fusarium* communities during malting were followed with PCR-DGGE (Polymerase Chain Reaction–Denaturing Gradient Gel Electrophoresis) and real-time PCR. Barley samples taken during processing were also analyzed for trichothecenes and for hydrophobins, also known as gushing factors. Furthermore, this study investigated the effects of heat-treatment on grain germination, gushing potential, enzyme activities and mashing performance. This study clearly showed that *Fusarium* growth could be effectively restricted by exposing the grains to steam prior to the steeping phase without influencing grain germination. Moreover, heat-treatment significantly reduced production of harmful *Fusarium* metabolites during malting. It inhibited mycotoxin formation and alleviated the gushing tendency. We also observed that restriction of fungal activities led to less extensive proteolysis and lower activities of xylanase and heat-stabile β-glucanase, as well as slightly lower wort separation. In order to balance the microbial communities in malting and to improve the process efficiency, heat-treatment could be combined with multifunctional microbial mixtures. Selective control of microbial populations with mild treatments in various steps along the barley-malt-beer chain could result in a successful strategy to suppress harmful organisms and to simultaneously enhance beneficial microbes contributing to malt modification and malt brewhouse performance.

O-22

The development and practical use of lipoxygenase-1-less malting barley with good brewing performance

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Lipoxygenase-1(LOX-1) is of great interest, because lipid oxidation, which is catalyzed by LOX-1, produces deteriorating substances in the brewing processes. Especially, trans-2-nonenal (T2N) is considered to be a major component of the off-flavor in aged beer, and trihydroxyoctadecenoic acid (THOD) is known to have an adverse effect on beer foam stability. Recently, we have discovered LOX-1-less barley lines which do not show significant LOX activity. The LOX-1-less malting barley was made by the introgression of the LOX-1-less character into the LOX-1-normal barley using the molecular marker assisted selection-mediated backcross breeding program. The brewing trials of the LOX-1-less malt indicated that the happoshu (Japanese low malt beer) brewed with this malt has improved flavor and foam stability. The purpose of the present study is to show the further advantage of the LOX-1-less malt when using another style of beer. To verify the superiority of the LOX-1-less malt, we performed brewing trials in a 50 or 400 liter scale pilot brewing plant. These trials included two types of the beer and two types of the happoshu. As a result, it was found that the T2N contents of the aged LOX-1-less beer (stored for 7 days at 37°C) were lower than that of the control beer. The sensory evaluations showed a noticeable superiority of the LOX-1-less beer in flavor stability. The amount of THOD in the LOX-1-less beer was lower than that of the control beer, which may reflect the superior beer foam stability (NIBEM value) of the LOX-1-less beer. We found no obvious changes between the LOX-1-less beer and control beer regarding the thiobarbituric acid index (TBI) value and the level of strecker aldehydes, which are indicators of aging caused by factors other than lipid oxidation. These results suggest the superiority of the LOX-1-less malt in brewing not only happoshu, but also other various types of beers. At present, we are producing a commercial LOX-1-less malting barley variety, and we expect that the LOX-1-less malt will contribute to progress in future brewing industries.

O-23

Thiol oxidase and the reason why we store malt

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A single thiol oxidase (molecular weight 35,700) has been purified from malt. The enzyme has a high pH optimum around pH 8.0 but only approx 20% of the activity at pH 5.5 and barely any if the pH is lowered to 5.0. It is relatively thermo-tolerant, with significant survival of activity even at 80°C. The enzyme is present in raw barley and decreases in activity during germination. The enzyme declines in activity during storage of malt, with complete decay in 2 weeks at 30°C and almost complete disappearance after 3–4 weeks at 20°C. It was demonstrated that thiol oxidase reduced filtration rates in gel protein systems. We propose that thiol oxidase-catalyzed oxidation of gel proteins in mashes is a key factor in lessening rates of wort separation. We further propose that the reason that malt storage leads to increased rates of wort separation is because there is a progressive decline in the amount of thiol oxidase in malt.

O-24

Application of alternate cereals and pseudocereals as a raw material with functionality for the brewing process and final beer

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In a worldwide view barley is the number one brewing cereal. Nevertheless there is a wide range of common and less common cereals (corn, em-

mer, einkorn, durum wheat, millets, oat, rice, rye, sorghum, spelt, teff, triticale, tritordeum, wheat) and pseudocereals (amaranth, buckwheat, quinoa), which can be successfully substituted for barley or barley malt as brewing material. Some of them, like rice, corn, millet and sorghum, are often used as brewing adjunct. Cereals like wheat, rye, oat, emmer, einkorn are sometimes used as adjunct or malt for special top fermented beers. All of them have a wide range of functions, like enhancing foam stability, antioxidative potential, stability of turbidity, color impact, advancing lautering process, increasing zinc content, increasing secondary plant ingredients (e.g. rutin), development of special flavor and flavor stability, upgrading microbiology stability and development of gluten-free beverages. This work focuses on these functions and shows the different functionalities of the different cereals and pseudocereals.

O-25

Malting and brewing with buckwheat—A gluten-free alternative

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Celiac disease (CD) is an inflammatory disorder of the upper small intestine and is caused by the ingestion of specific cereal storage proteins in genetically susceptible individuals. Current studies show that approximately 0.9 to 1.2% of the Western population suffers from this affliction. For those who suffer from CD, the only safe alternative is to avoid the ingestion of gluten-containing or gluten-contaminated food and beverages, resulting in a need to develop alternatives, for example, to beer based on barley malt. This presentation gives an overview of a novel approach for the development of gluten-free buckwheat malt and its subsequent use in the brewing process. Buckwheat (*Fagopyrum esculentum*) is a pseudocereal from the family Polygonaceae. It is a rich source of starch and contains many valuable compounds, such as high quality protein and polyunsaturated fatty acids, antioxidant substances and dietary fiber and, therefore, is recommended as an ingredient for functional food products. Furthermore buckwheat is gluten-free and, therefore, acceptable for the diet of CD sufferers. This presentation comprises a detailed characterization of buckwheat and its assessment as a potential raw material for malting. For this, optimization of malting parameters in terms of improving typical malt-quality characteristics (e.g. fermentability, extract, free amino acids, viscosity, etc.) using mathematical modeling is discussed in detail. Furthermore, variances of some functional constituents as well as the detection of ultra structural changes in the endosperm using advanced microscopy during malting are shown. Special emphasis is placed on the brewing process, which was performed on a 1000 L pilot scale plant with 100% buckwheat malt. A full flavor analysis combined with a sensory evaluation of the final beer was performed. Processing problems with saccharification, mash filtration and clarification during beer maturation, as well as beer filtration, due to the specific characteristics of buckwheat malt, like high gelatinization temperature, high viscosity, low enzymatic activity and flat husks, were expected. Results show that the low amylolytic potential of buckwheat malt could be overcome without problems by the use of industrial brewing enzymes. Furthermore, mash filtration and clarification during beer maturation could be enhanced by the addition of rice husks and viscosity lowering enzymes. However, it is necessary to recognize that further optimization of buckwheat malting is required to achieve higher cytolyses. Notwithstanding these issues, it is expected that once the processing problems have been overcome, buckwheat beer will find acceptance with time, particularly among those who suffer from CD.

O-26

Functional components in malting and brewing

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Nowadays our nutrition is mainly based on only three cereals, wheat, rice and corn, they contribute over 75% to the world's starch production. At the same time diseases caused by wrong or unbalanced diet are becoming a severe problem in Western countries. In this regard the enrichment of functional components in the malting process with the objective to provide their beneficial health effects to the consumer is a very important field. The malting process is influenced by the quality of the raw material and by several process parameters (e.g. moisture, temperature and time), and the enrichment of functional components depends on the same variables. To reduce the necessary number of trials for the evaluation of the optimal conditions for enrichment of various components we use software for the "design of experiments" (DOE). This software (Design Expert, Stat-Ease Inc.) supports several different statistical approaches, like various "Factorial Designs" or "Response Surface Methods" (RSM). RSM was developed for process optimization in technical fields, but it has been successfully used in various biotechnological applications including brewing applications. With RSM the interactive effects of various process conditions are modeled empirically. One group of the functional components we investigated are the arabinoxylans. This type of dietary fiber is known to provide health benefits. In order to investigate this group of substances it was necessary to establish a method for the determination of water-soluble and water-insoluble arabinoxylans. The method we used consists of acidic hydrolysis of the arabinoxylans, followed by HPAEC/PAD detection. Using this method we were able to enrich water-soluble arabinoxylans in wheat malt. Other methods we used are AOAC Method 991.43 for the determination of total and soluble dietary fiber and AOAC Method 999.03 for the determination of fructans and oligofructose. Another interesting group of components are the flavanols. We established a method for the characterization of flavonols like rutin, vitexin and quercetin by HPLC separation, and the technique we used for the specific detection of catechins and procyanidins is based on post-column derivatization. This method has helped us to determine optimum malting parameters for the enrichment of these functional components in buckwheat malt. Additionally changes in the vitamin B_1 and B_2 content of cereals were monitored during the whole germination and malting process of different cereals as well as the changes in water-soluble arabinoxylan and fructan.

O-27

Comparison of iso-α-acid sensory thresholds obtained via a change-point model and standard ASTM methods

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This study compared proposed threshold values of iso-α-acids (iso) and tetrahydro-iso-α-acids (tetra) collected by time-intensity methods to threshold values determined by the ASTM 1432 ascending forced-choice method. The difference threshold, or just noticeable difference, is the amount of change in concentration from a constant stimulus that can be reliably identified by an observer. A trained panel evaluated seven concentrations of iso and tetra in an unhopped lager by a time-intensity procedure. Concentration dependent attributes were peak intensity, duration, area under the curve, and decreasing area. Two lines were fit within a compound to the panelist's per concentration-dependent data. This included a flat line to a change-point and a positively sloped line after a change-point. At concentrations greater than the change-point, the panelists could reliably detect changes in the attribute due to increases in concentration. Therefore, we hypothesized that the change-point value represented a difference threshold for the compound in the unhopped lager beer. Thresholds, according to the ASTM 1432 ascending forced-choice method, were collected for tetra and iso in the unhopped lager beer, and the change-point values of iso and tetra for each of the time-dependent attributes were compared to these threshold values. Eleven panelist's data were compared. One-sample *t*-tests within panelist were used to compare the per-panelist threshold values to the change-point concentrations per compound ($\alpha = 0.10$). Accordingly, the change-point parameter did not adequately predict the threshold value as measured by the forced-choice method for either iso or tetra in the unhopped lager beer.

O-28

Psychophysical models for the visual perception of beer

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The visual perception of beer by the consumer undoubtedly affects their expectations, even prior to consumption. Of the three distinct visual qualities of beer—color, clarity and foam—we have been evaluating descrip-

tive language and developing psychophysical models to assess color and clarity, and their interdependence. All visual assessments were carried out under validated control conditions, using a standard illuminant mounted in specifically-designed viewing cabinets (VeriVide®, Enderby, UK). Viewing models were developed using a range of standard solubilized colorants and cloudifiers (Unilever, Sharnbrook, UK; Sensient Colours, Kings Lynn, UK) suspended in aqueous media. The results showed that observers can be trained to accurately scale 'clarity' and color appearance of liquid products. It was also found that perceived color appearance is affected by different levels of cloudifiers. Models relating visual assessments and physical measurements are being developed, and these can be applied to facilitate our understanding of how consumers interpret such visual cues in their appraisal of product quality.

O-29

A new global approach to tasting

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With the large-scale globalization of our company, a decision was made to use a common method of tasting in all countries. This provided an opportunity to review the various systems that were currently in use and to look at their advantages and disadvantages. A new approach was developed that has provided a number of distinct advantages over the old systems. These include the ability to use the full range of the taste scale; a capability to track trends and improvements; the creation of a problem-solving tool; and an auditable system as well as a means of benchmarking a tasters performance with peers worldwide. Brands produced in different locations around the world can be rated in their centers of production knowing that the values obtained are comparable to those using a central panel without the need for transporting samples. The rationale behind this new system will be discussed together with the benefits achieved.

O-30

The bitter, twisted truth of the hop

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In the last 50 years, hop chemistry has been profoundly transformed by Miller Brewing Company to advance brewing technologies to higher quality, increased consistency, and improved economics. This can be attributed to the evolution of hop chemistry. The advent of liquid/supercritical CO_2 extraction renders an organic-solvent-free process for development of downstream products. The development of advanced hop products invalidates the International Bitterness Units (IBU) method for determining bitterness quality, obsoletes the co/n ratio of α-acids for foam potential, and improves light and flavor stability in beer. Exploiting the water soluble substances in hops uncovers flavoring glycosides as the true hop flavor precursors and polyphenols for enhancing the flavor stability of beer.

O-31

Anti-staling effects of hop-polyphenols on lager beer flavor

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Hop-derived polyphenols may increase potential staling resistance of lager beer due to their antioxidative nature. This study gives preliminary insight of the impact of hop-derived polyphenolic extract on lager beer staling during accelerated storage, the results of which will benefit the brewing and hop industry by increasing knowledge of beer flavor stability and adding value to a waste stream (spent hop material). A polyphenol-rich extract was isolated from spent Galena hop material via column chromatography (Amberlite FPX66 resin) and dosed at 100 ppm into a commercial lager beer (Coors Original) (PP). Beer with and without added polyphenols was bottled, pasteurized (60°C, 10 min, ≥25 PU), and force-aged in the dark at 21°C and 29°C. Samples were pulled weekly and stored at 1°C until analysis. Antioxidant power of the samples was assessed chemically via FRAP, DPPH and EPR indices while qualitative staling effects were assessed by a trained panel according to a descriptive analysis sensory protocol. For sensory evaluation, seven trained panelists participated in the study, each completing 3 replications per sample over 9 total testing sessions. The descriptive ballot was based on a consensus of six aroma descriptors and three basic/mouthfeel descriptors that were rated on a 16-point intensity scale (0 = none, 15 = extreme intensity). Data were analyzed according to a partially randomized block design. Chemical analysis confirmed an anti-staling effect of dosed PP as measured by FRAP, DPPH and EPR. Metal analysis (ICP-OES) demonstrated that PP treatment reduced total Cu and Fe by10 ppb at week 0. Force-aging the PP dosed beer did not seem to significantly affect total metals at either storage temperature. Sensorially, beers treated with PP were statistically different from beers which did not receive polyphenols (No-PP) for all but one aroma descriptor ($p \leq 0.01$). However, a significant interaction effect (polyphenol-by-panelist) resulted for each descriptor ($p \leq 0.01$). More importantly a significant temperature effect for (reduced) cardboard aroma was seen in the PP treated beer stored over 6 weeks at higher temperature.

O-32

Influence of harvest date, growing location and sub-variety on the composition of Golding hop essential oil

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Goldings, first developed in the 1700s, consist of a group of traditional English hop varieties for which the exact origin is unknown. Over the past century at least six sub-varieties have been grown which are all marketed as "Goldings". These are cultivated both in the Kent and West Midlands growing areas of England, and these distinct geographically areas have very different climate patterns. Over the last three years hops from all sub-varieties and growing areas have been harvested at different dates over a three-week window to determine the effect of these parameters on the level and composition of the essential oil, particularly those components considered to be character impact molecules. It has been possible through this study to recommend an optimal harvest date for this variety and to provide supplementary information to brewers when they select hops each year. A better understanding of the composition of each variety has also enabled a wider choice of hops to be made available at a time of limited supply.

O-33

Examination of the flavor potential of glycosidically bound flavor compounds from hops

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At the 2006 ASBC annual meeting, we presented results on the flavor potential of hop glycosides and the influence of yeast. It became clear that acid and enzymatic hydrolysis of hop glycosides, extracted from spent hops after supercritical CO_2 extraction, led to fruity, floral, herbal and/or spicy flavors. Both *Saccharomyces* and *Brettanomyces* brewing yeasts showed a glycoside hydrolase activity towards hop glycosides. To gain more insight into the flavor potential of hop glycosides, a more thorough examination of released hop aglycones was carried out. Well-known (e.g. linalool, geraniol) and less typical (e.g. methyl salicylate) hop volatiles were identified. The compounds dihydroedulan I and II (elderberry-like aroma) and theaspirane A and B (woody- and camperous-like aromas) increased after enzymatic hydrolysis, and were identified for the first time in hops and in beer (dry hopped). These norisoprenoids are most likely formed by partial acid-catalyzed cyclization of precursors which occur as glycosidic compounds. Further, the flavor potential of hop glycosides was evaluated in wort fermentations in which a hop glycoside extract was added at different ratios. Release of hop volatiles and the glycoside hydrolase activity of yeast were followed during the fermentations. The use of different *Saccharomyces cerevisiae* strains showed that exo-1,3-β-glucanase activity led to a moderate increase in certain hop aglycones, like 1-octen-3-ol and dihydroedulanes. The use of a mixed culture of *Saccharomyces cerevisiae* and *Brettanomyces custersii* led to a remarkably higher release of hop volatiles and demonstrated the flavor enhancing properties of the β-glucosidase activity of *B. custersii*. Here, formation of both the newly identified hop volatiles dihydroedulanes and theaspiranes was observed. Sensory analysis showed that an increased addition of hop glycosides led to a higher general appreciation. The acquired

knowledge can be applied in methods to utilize the flavor potential of hop glycosides more efficiently to obtain beverages with an improved and refined flavor or to create new types of beverages.

O-34

Adding technical value to the 21st century brewery

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The traditional brewery laboratory has focused mainly on the ingredient and product analysis, as well as research, that ultimately could improve beer quality. In the first decade of the 21st century there has been more emphasis on the brewing laboratory adding value to the business. One response to this mandate is to adopt a solution-focus that emphasizes practical process control, rather than end-product testing or basic science focus. An example of applying such an approach to the control of beer flavor will be discussed in detail. First, the potential critical control points were identified, then confirmed through lab research and brewery trials. Next, in-process measures that can be used to monitor the process were established. Finally, ranges for the critical control points and measures were specified. It is also important to capture this knowledge since it can be lost as personnel leave: a web-based tutorial was developed to address this.

O-35

A survey and explanation for the variation in the levels of diastatic power enzymes that indicate potential malt fermentability

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In this study over 1000 commercial malting samples from Australia and internationally, primarily malted in 2005 and 2006, were analyzed for their levels of the DP enzymes α-amylase, β-amylase and limit dextrinase. The survey showed that there was more variation within the varieties for DP and DP enzymes than between varieties. The data was evaluated, and a micro malting experiment was conducted to ascertain if the wide range of malt qualities observed were the result of customer specifications, environmental conditions under which the barley was grown, variety or malting practices. The evaluation of malt used by two breweries over the course of a year suggested that the conventional brewery customer specifications for variety, KI and DP are somewhat successful in constraining potential fermentability variation. The conditions under which barley for malting was grown were also plausible factors that could explain the observed differences in DP enzyme levels. However, micro-malting barley sourced from different regions showed that malting conditions had a strong influence on the malt levels of α-amylase and limit dextrinase. Combined, the observations and conclusions of this study further support our previous recommendations that the routine measurement of the individual DP enzymes would most likely improve the consistency and predictability of the potential fermentability of malt supplied to brewers. The manuscript for this potential presentation was submitted for publication to the *Journal of the American Society of Brewing Chemists* in December 2007.

O-36

Ethanol and sucrose interaction cross-products and influence on specific gravity and refractive index

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In 1830 the French chemist M. Emile Tabarie introduced the hydrometer, and a procedure for boiling off wine spirits in an uncovered vessel. The alcohol concentration in the wine was back-calculated based on the difference in specific gravity between the wine SG and the residue SGE brought back to its original volume. The Tabarie equation: SGA = SG – SGE + 1, and his patented apparatus relied on tables used to convert SGA to alcohol % by volume in the virtual distillate and hence the wine. Today direct measurements of alcohol which utilize gas or membrane sensors or NIR spectroscopy no longer depend on specific gravity to measure alcohol. But Tabarie's equations remain essential for inferring real extract or estimating apparent extract/SG when only alcohol and extract are known. Water, alcohol, and polysaccharides participate in intermolecular hydrogen bonding due to the dipole-dipole attractions between hydroxyl groups. Tables which convert SGA to ethanol and SGE to sucrose concentrations account for single aqueous-solute relationships, but do not consider ethanol-sucrose interactions or the disruptive effects that a second solute has on the remaining intermolecular forces. To elucidate these interactions a series of 124 sucrose and ethanol solutions with a combined weight up to 35% were measured in triplicate for SG and RI. OLS regression and cross products of SGA and SGE were used to model the difference between the experimental SG's and a gravimetric Tabarie. Adj.R2 = 0.9994, SE = 4.4E-5, n = 124. For a solution of 5% ethanol and 5% sucrose by weight, the model is lower than the Tabarie by mass –2.4E-4 and higher than the Tabarie by volume +1.0E-4. Additional trials using beer distillation products confirmed the model. It was also determined that the scale refractive index was simply a linear combination of the component ethanol and sucrose SRI's. Adj.R2 = 0.9993, SE = 1.5, n = 70. Collaborative data from LGC/BAPS and ASBC/BACK indicates that sucrose SRI's modeling real extract were 2.9% lower, and ethanol SRI's modeling beer alcohol were 4.3% lower and 7.6% lower then the measured beer SRI's for LGC and ASBC respectively.

O-37

Fourier-transform infrared (FT-IR) spectroscopy, a cost-effective and high-resolution method to identify, differentiate and monitor wild and cultured yeasts in a brewing ecosystem

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In brewing microbiology bacteria and yeasts are of major interest as spoilage and culture organisms. Methods like real-time PCR, rRNA micro-FISH and RNA-hybridization probes to detect and identify beer spoiling bacteria are already established in many breweries to control the presence or absence of beer spoiling bacteria in the production chain. The monitoring and identification of wild and brewing yeasts still is an underestimated topic. Several culture-based, fermentation spectra and DNA-based methods are proposed in the literature but most of them are time-consuming, cost-intensive and are mostly developed for one special field of application (e.g. differentiation of brewing strains). The aim of this study was to analyze the potential of Fourier-transform infrared (FT-IR) spectroscopy to identify wild yeasts and to differentiate culture yeasts including ale and lager strains. The FT-IR spectroscopy was introduced as a technique to identify microorganisms by the group of Dieter Naumann, and it has gained growing interest for identifying microbes on the species and strain levels. The absorption of infrared light by cell components results in fingerprint-like spectra which reflect the overall chemical composition of the cells under investigation. As a standardized physico-chemical technique, FT-IR spectroscopy benefits from the fact that operating costs are extremely low, as practically no consumables are required, while at the same time spectra contain a huge amount of information, which can be exploited to help solve different kinds of identification problems. By comparison with large reference data sets, spectra of microbial cells can be analyzed for identification purposes, or to reveal certain characteristics or even strain identity. The results of the present study confirmed that the general benefits of FT-IR spectroscopy could be transferred to the investigation of a brewing ecosystem. An already existing yeast FT-IR spectra database was expanded using spectra of yeast strains isolated in different breweries and industrial strains from culture collections within a period of 18 months.

O-38

Production of hydrogen sulfide during secondary fermentation related to pH value

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Lager yeast is known to produce higher levels of sulfur compounds, such as hydrogen sulfide (H_2S) and sulfite, than those of ale yeast. In particular, "happoshu" and the so-called "third category beer", which are Japanese beer-flavor beverages brewed with little or no malt content, show higher levels of H_2S and also lower pH values than those of regular beer. At the

71st ASBC Annual Meeting in Victoria, BC, Canada, based on the results of the gene expression analysis of the two types (*Saccharomyces cerevisiae* and *Saccharomyces bayanus* types) of genes, we showed that the gene expression balance of *MET3* and *MET10* leads to higher levels of sulfite being produced in the lager yeast. However, it has not been fully clarified whether the lager yeast produces a higher level of H_2S. During our further study of Japanese beer-flavor beverages, we found that H_2S was detected, in the secondary as well as the main fermentation. Furthermore, the content of H_2S produced during the secondary fermentation tended to increase further at low pH values. In this report, we investigated the correlation between the amounts of H_2S and pH values during the secondary fermentation related to sulfite. Sulfite is one of the intermediates of sulfur amino acid biosynthesis. Although sulfate is generally used as a source of sulfur in wort, sulfite can be utilized only at low concentrations. Sulfite exists as a mixture of three forms (SO_2, HSO_3^- and SO_3^{2-}) depending on the pH value, and the yeast can uptake sulfite only in its molecular state (SO_2) by simple diffusion (1) since the yeast membrane causes the anion to become electrically charged, and the ratio of SO_2 would be higher in fermenting wort as its pH values subside. The results of adjustment trials of the pH value within the range 3.0 to 5.0 during secondary fermentation indicated a negative correlation between the contents of H_2S and the pH values, and the sensory test also showed the same pattern. This suggests that the increase of H_2S level during the secondary fermentation would be due to the form of sulfite depending on the pH value and that the increased H_2S content of Japanese beer-flavor beverages may be responsible for the low pH values. Based on this result, we propose a new model for the production of H_2S during secondary fermentation by the simple diffusion of sulfite. Reference: 1) Malcolm Stratford and Anthony H. Rose. *J. Gen. Microbiol.* 132: 1-6, 1986.

O-39

High cell density fermentations: Promises and challenges

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In the traditional production of lager beer, the fermentation process takes about 1–2 weeks before entering a maturation period of 1–3 weeks. As a consequence, fermentation and maturation are the most time-consuming steps in the production of beer. To improve the productivity of the beer fermentation process, several strategies can be adopted. The interest in immobilized yeast for primary beer fermentation seems to have dropped, but the essence of this technique was to improve productivity by maximizing the cell concentration in the reactor. Therefore, a promising strategy could be the increase of suspended yeast cells in the fermentor. In a first experiment, different pitching rates (10-20-40-80-120 million cells/mL) were applied in tall tubes (2 L) to investigate the influence of this variable on yeast physiology and beer quality. The fermentation speed was drastically increased when higher initial cell concentrations were used. The net growth (maximum cell concentration – initial cell concentration) decreased with increasing pitching rate, which indicates that there must be a growth limiting factor when using higher pitching rates. It was hypothesized that the depletion of oxygen, needed for the formation of essential membrane compounds, could be the limiting factor for yeast growth at higher pitching rates, because less oxygen per cell will be available. Throughout the fermentations, important physiological parameters of the yeast were monitored, such as viability, acidification power, glycogen, trehalose and fatty acids. The results of these analyses revealed that physiological differences exist between normal and higher pitching rates, although it cannot be stated that higher pitching rates result in a poorer yeast condition. In a second experiment, the yeast oxygenation conditions were varied in the high cell density fermentations (80 million cells/mL) and consequently altered growth profiles were observed. In addition, high cell density fermentations were carried out at pilot scale, and after reaching 80% attenuation, the beers were chilled and GC headspace analysis was performed. No clear correlation was observed between the higher alcohol/ester ratio and the pitching rate or oxygenation condition which was used. In addition, the outcome of tasting trials of the different beer types, performed by a professional panel, showed no significant differences. These findings show that the use of high cell densities in beer fermentations provides promising opportunities, although challenges for this technique remain to be investigated, such as the impact of high cell density fermentations on the fermentation performance of different yeast generations.

O-40

Effect of the fermentation process on staling indicators in order to influence the flavor stability of beer

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Consumers consider flavor as the main quality parameter of beer. However, the flavor profile is subject to changes during storage due to many kinds of chemical reactions. As a beer ages, fresh flavor notes diminish and several typical aged flavors appear. This lack of flavor stability is of great concern for brewers as it is important that a commercial beer is consistent and satisfies the expectations of the consumer at all times. Despite extensive research, it remains very difficult to control flavor stability. Since the fermentation process has an enormous impact on many aspects of beer, it might also influence flavor stability considerably. Biochemical processes that occur during fermentation are not only responsible for flavor formation due to the production and removal of flavor compounds, but they might also influence flavor stability in several ways. Although it has already been shown that the flavor stability is different for beers produced with different yeast strains, research on this effect is very limited. In this work, the effect of yeast strain selection on staling indicators was studied in order to influence flavor stability. Ten top fermenting *Saccharomyces cerevisiae* yeast strains were compared in lab scale fermentations. The effect of yeast on several parameters known to influence flavor stability were evaluated. Additionally, volatile flavor compounds were analyzed with headspace SPME GC-MS. The concentration of flavor compounds able to mask aged flavors, sulfite content and pH of the resulting beers were found to be yeast strain dependent. Next to this, the reducing power of yeast appeared to be especially interesting. A clear effect on several volatile carbonyl compounds and a substantial reduction of precursors of aging reactions was observed. As these factors have a considerable influence on flavor stability, the reducing power of yeast was studied in more detail on lab and semi-industrial scales. Two yeasts were selected for the fermentation of wort with and without addition of volatile carbonyl compounds on a lab scale. Additionally, these yeasts were used for the fermentation of wort with low and high contents of volatile staling compounds and precursors on a semi-industrial scale. The reduction of several volatile carbonyl compounds was substantial and resulted in beers with a similar content. Afterward, the beers were aged, and flavor stability was evaluated by monitoring the evolution of volatile staling compounds and by sensory analysis.

O-41

Managing large capacity cylindroconical fermentors, correlations between physical parameters, yeast dispersion, yeast metabolic activity, fermentation performance and beer quality

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Fermentation has a critical influence on overall brewing process efficiency and beer quality. The international brewing market is fiercely competitive, and in response many brewers have sought methods for maximizing fermentor output. Strategies include the use of large batch sizes fermenting high gravity worts at relatively high temperatures. All of these tend to increase the stresses to which brewing yeast is exposed. The most popular choice of fermenting vessel design is cylindroconical. Apart from improved hygiene and increases in capacity, the design and operation of these vessels has changed little since their introduction in the late nineteenth century. The regulation of important variables such as temperature, pitching rate, wort concentration and dissolved oxygen can now be achieved with acceptable precision and reproducibility; however, the actual values that are chosen for these parameters are based on empirical observation. The assessment of conditions within the fermentor is typically based on single point measurements or discontinuous analysis of off-line samples. This infers homogeneity of vessel contents. Variables such as fermentor aspect ratio, capacity and the method and time of filling, which

are all known to have dramatic impacts on process efficiency and beer analysis, may be based on considerations that have little to do with the biological process that is occurring within them. For example, aspect ratio and capacity are often based on the sizes of cylindrical sections that are readily available for fabrication into finished vessels as well as the ease of transport into and space available within the brewery. Replacement of redundant plants with more and larger fermentors without a concomitant uprating of brewhouse capacity is not uncommon. This presentation will review and consolidate two strands of research. Previously published work has examined the distribution of yeast within high gravity lager fermentations performed within a cylindroconical vessel with a working capacity of 1600 hl. The results have shown that there is significant heterogeneity for most of the fermentation. The degree of heterogeneity is dependent on the nature of the yeast and the fermentation management regime. Advances in knowledge of the yeast genotype and the factors which control its expression now allow a much more accurate prediction of yeast behavior under the conditions to which it is exposed during brewing fermentations. In particular, it is possible to use metabolic triggers such as oxygen availability to control conditions during wort collection to ensure that important beer flavor components such as esters are synthesized in desired amounts during the subsequent fermentation. Armed with this knowledge it is possible to suggest more appropriate procedures for fermentation management which safeguard yeast health, provide shorter and more predictable cycle times and result in beer with a consistent and desired composition.

O-42

New product development strategies for brewers

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Brewers of all sizes are spending increasing time and resources developing and launching new beers. Whether focused on traditional or cutting-edge beer styles, brand extensions or radical category-redefining hybrid beverages, a structured and thoughtful new product development process can yield tremendous benefits in terms of the use of resources and speed to market. A process that is additionally focused first on understanding your consumer will always increase the chances of a successful product launch and long term potential. Ideas are cheap and easy—and abundant. Applying the appropriate filters to weed out the merely good ideas and then further refine the great ideas is the goal. Development methodologies should have an emphasis on those steps most often ignored—those which can and should occur well before a single kernel of malt is milled to test a prototype recipe. Consumer-centric idea generation, the application of concept screens, testing and refinement of product ideas, and the use of a "gated" approach all lead to more effective use of limited resources. This is true whether those resources are small (and constrained) or vast (and constrained). The design of consumer taste-testing and the use of consumer feedback in prototype development and recipe refinement can yield important insights if properly executed—and entirely misleading results otherwise.

O-43

The sensory directed product development of Presidente Light: A success story in the Dominican Republic

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Developing a successful new beer has many challenges. This presentation will describe the sensory directed process Cerveceria Nacional Dominicana and GEI used to meet these challenges in the development of Presidente Light. The first step was to benchmark existing beer flavor in the Dominican market using flavor maps generated by professional sensory panelists. The next step was to understand consumer needs for beer flavor. A professional sensory panel selected a group of beers that represented a wide range of flavors to be used in the consumer test. The moderator for the consumer sessions was trained to use a unique facilitation approach to get participants to score overall liking, generate terms, identify key drivers of flavor, and rate the beers on their key drivers. In addition, participants were asked to score both their favorite beer and their ideal beer. The same beers assessed by consumers were also evaluated by a professional sensory panel using standard flavor attributes. The combination of consumer and professional scores for each sample allowed us to interpret consumer responses into actionable information. In addition, we were able to model consumer responses to predict overall liking for future beers, as well as optimize individual flavor characteristics. Next, we used the consumer and professional flavor data to identify distinct flavor segments on our market beer flavor map. We determined which existing beers fell into existing flavor segments by plotting them on the same map. We were then able to identify new flavor opportunities in segments with few, or no, existing beers. We were also able to estimate the potential size of each flavor segment by using consumption data collected in the consumer test. Last, the professional sensory panel was used to evaluate the flavor of a set of prototype beers, plot them on the flavor maps, and determine if they fell in flavor segments that were of interest. Confirmation consumer tests were then conducted, and the final beer was selected and launched under the brand name Presidente Light. Presidente Light quickly gained significant market share and in less than two years has obtained over 50% of the Dominican beer market.

O-44

Withdrawn

O-45

Development of detection medium for hard-to-culture beer spoilage lactic acid bacteria

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Beer spoilage lactic acid bacteria (LAB) are generally difficult to detect using culture media. Among beer spoilage LAB, *Lactobacillus paracollinoides* and *Lactobacillus lindneri* strains are found in hard-to-culture states upon primary isolation from brewery environments and often cause microbiological incidents without being detected by quality control tests in breweries. Nevertheless, detection media have often been evaluated with easy-to-culture beer spoilage LAB strains that are maintained in nutrient-rich laboratory media, an environment considerably different from those found in nature. This study therefore aimed to acquire hard-to-culture beer spoilage LAB as bioresources and develop a medium for detecting these groups of microorganisms. Four hard-to-culture beer spoilage LAB strains, belonging to *L. paracollinoides* and *L. lindneri*, were obtained by repeatedly subculturing the wild-type strains in beer. In contrast to the wild-type counterparts, these beer-adapted strains were found to be hardly detectable on MRS agar, a typical medium for detecting beer spoilage LAB in the brewing industry. To develop a countermeasure against these hard-to-culture beer spoilage LAB, a beer-based medium was modified. As a consequence, the supplementation of a small amount of MRS medium was found to enhance the growth of the hard-to-culture beer spoilage LAB strains obtained in this study. In addition, sodium acetate was shown to improve the selectivity of this beer-based medium. Further comparative study was performed with five other media widely used for the detection of beer spoilage LAB in the brewing industry. This experiment revealed that the newly developed medium, designated ABD medium, possessed superior sensitivity for hard-to-culture beer spoilage LAB and comparable sensitivity with easy-to-culture beer spoilage LAB. Moreover, ABD medium was found to suppress the growth of nonspoilage microorganisms and thereby allow the selective growth of beer spoilage LAB, a feature not observed with other detection media. These results suggest that the detection by ABD medium can be used as an indicator for differentiating the beer spoilage ability of LAB without additional confirmatory tests in breweries. Further field studies with ABD medium revealed that more than half of the test samples collected in this study contained hard-to-culture beer spoilage LAB, suggesting considerable proportions of beer spoilage LAB are in hard-to-culture states in beer and related environment. Taken together, ABD medium is considered an effective tool for comprehensive detection of beer spoilage LAB in breweries.

O-46

Rapid detection and identification of beer spoilage lactic acid bacteria by microcolony method

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In the brewing industry, microbiological quality of beer products has been traditionally ensured by culture methods, which detect colonies grown on selective media. Although regarded as a reliable approach for microbiological quality control (QC) in breweries, these methods are time-consuming and require additional confirmatory tests before any corrective actions are taken. In order to reduce the time for microbiological QC tests, several rapid alternatives have been proposed, including the fluorescence *in situ* hybridization (FISH) technique. Since the FISH method directly detects beer-spoilage bacteria with a species-specific fluorescein-labeled probe targeted to rRNA, it enables us to identify contaminants in a species-specific manner without culturing. The FISH method also shows sensitivity sufficiently high to detect a single beer-spoilage bacterial cell. Nevertheless, due to the presence of false-positive noise signals, it may require further confirmatory tests for the definitive interpretation of test results to be made. To solve this problem, we evaluated a microcolony method for the detection and identification of beer-spoilage lactic acid bacteria (LAB). In this approach, bacterial cells were trapped onto a polycarbonate membrane filter and then cultured on ABD medium, a medium that allows highly specific detection of beer-spoilage LAB strains. After a short-time incubation, viable cells forming microcolonies were stained with CFDA and counted with the μFinder inspection system. As a result, all of the beer-spoilage LAB strains examined in this study were able to be detected within three days of incubation. The specificity of this method was found to be exceptionally high and even discriminate intra-species differences in the beer-spoilage ability of LAB strains. These results indicate that our microcolony approach allows rapid and specific detection of beer-spoilage LAB strains with inexpensive CFDA staining. For further confirmation of the species status of detected strains, subsequent treatment with species-specific FISH probes was also shown as effective for identifying CFDA-detected microcolonies. In addition, no false-positive results arising from noise signals were recognized for the CFDA-staining and FISH methods, because of the comparatively much stronger signals obtained from microcolonies. Taken together, the developed microcolony method was demonstrated to be a rapid and highly specific countermeasure against beer-spoilage LAB and compared favorably with the conventional culture methods.

O-47

Agar gradient-plate technique for determining beer-spoilage ability of *Lactobacillus* and *Pediococcus* isolates

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To date, identification of beer-spoilage bacteria has largely taken two approaches: identification of specific species of bacteria regardless of ability to grow in beer or the identification of spoilage-associated genes. The dilemma with these methods is that they are either overly inclusive (i.e., detect all bacteria of a given species regardless of spoilage potential) or overly selective (i.e., rely upon individual, putative spoilage-associated genes). As such, our goal was to design a method to assess the ability of bacteria to spoil beer that is independent of speciation or genetic background. Our solution to this problem is an agar gradient-plate technique. A gradient is created by pouring a base layer of MRS agar containing hop-compounds on a slant in a square Petri plate. Once solidified, the Petri plate is laid flat, and MRS agar is poured on top to create a layer through which the hop-compounds must diffuse. Bacterial isolates are stamped onto the plate using the side of a glass microscope slide and growth of isolates along the gradient of hop-compounds is measured to determine resistance. Through the development of this assay, we have made the additional finding that the basis for the ability to grow in beer differs for *Lactobacillus* and *Pediococcus* isolates. In contrast to *Pediococcus* isolates, hop-resistance alone is not optimal for identification of *Lactobacillus* beer-spoilage ability (76–82% accuracy in isolates tested). Instead, the presence of ethanol (added to a concentration of 5% to both layers of MRS agar), in addition to hop-compounds, is necessary for accurate prediction of the ability of *Lactobacillus* isolates to grow in beer (100% accuracy in isolates tested). In several instances, addition of ethanol to the agar gradient plates produced an enhanced resistance of beer-spoilage *Lactobacillus* isolates to hop-compounds, possibly due to an induced change in membrane permeability. The opposite effect of ethanol was also observed, but only for bacteria unable to grow in beer. Testing of the gradient plate technique was performed on 85 *Lactobacillus* and 50 *Pediococcus* isolates and was highly accurate in differentiating between isolates capable of growing in beer and benign bacteria (chi-square $P < 0.0005$) in only 36 hours. Our agar gradient-plate technique provides a rapid and simple solution to the dilemma of assessing the ability of *Lactobacillus* and *Pediococcus* isolates to grow in beer and provides new insights into the different strategies used by these bacteria to survive under the stringent conditions of beer.

O-48

The practical hints for brewing from premature yeast flocculation (PYF)-positive malt

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Premature yeast flocculation (PYF) is a serious problem in the brewing industry. If the beer is brewed with PYF-positive malt, yeast flocculate prematurely during fermentation while high sugar concentrations remain and lead to a marked reduction in the number of yeast cells. As a result, a high level of vicinal diketones (VDKs) remains in the beer. Many researchers all over the world, including our laboratory, are researching PYF, but the cause of the PYF phenomenon is still unresolved. Therefore, we investigated the brewing method for PYF-positive malt, because there is a possibility that PYF-positive malt is supplied to our breweries. Considering the mechanism of formation and reduction of VDKs with yeast during fermentation and maturation, we planned two tests: green transfer (Gruenes Schlauchen) test and high temperature fermentation and maturation test. These tests were conducted with a 5-kL pilot-plant, mixture of PYF-positive and -negative malt (4:6). We evaluated these tests based on the number of yeast cells, the value of VDKs, and the finished beer tasting. As a result of the green transfer test, the number of yeast cells during maturation increased, the value of VDKs decreased, and the beer taste improved. In the high temperature fermentation and maturation test, yeasts flocculated during maturation; however, VDKs reached a low value, and the beer flavor improved. These technologies are applied to our breweries and certainly make it possible for us to brew a better beer from PYF-positive malt than before, but we have just overcome the first hurdle of PYF-positive malt brewing. We still have many technical difficulties to overcome these PYF problems.

O-49

Factors that promote premature yeast flocculation condition in malt

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Flocculation is the process whereby yeast aggregate and sediment from solution at the end of fermentation. The premature flocculation of yeast during fermentation whilst the fermentable sugar concentration remains high results in a marked reduction in the number of yeast cells in suspension, leading to incomplete fermentations, and can also extend the time required for conditioning and maturation. Although premature yeast flocculation (PYF) is a sporadic phenomenon, it is an increasingly important quality issue, and some brewers are setting malt quality targets for this parameter. PYF is widely considered to be induced in the field when barley

is grown under sub-optimal conditions, resulting in the formation of an antimicrobial agent in response to fungal attack, but little is known about its subsequent control or remediation. There is also little knowledge concerning the possible influence of malting conditions on the development of the PYF condition. The results of research investigating the correlation between malting process parameters and the suppression or development of the PYF condition in finished malt are presented and discussed.

O-50

Improvement of premature yeast flocculation (PYF) caused by PYF-malt using tannic acid

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Premature yeast flocculation (PYF) is a phenomenon whereby factors in malt (PYF-malt) cause yeast to flocculate during fermentation when fermentable sugar is still present in the wort. As a practical countermeasure, breweries could consider adding a blend of non-PYF malt; however, there are limits to the volume of non-PYF malt that could be blended in, and the resulting long-term storage of PYF-malt in the brewery would lead to long-term silo occupation and to degradation in malt quality. In this study, we noted that PYF improved with the addition of gallotannin or other tannic acids to the wort or yeast. To examine this phenomenon, we have conducted several fermentation trials by changing the amounts of tannic acids added, the timing of addition, and the type of tannic acids used. Our results indicated that the addition of tannic acid at appropriate levels (under 4.0 g/kg malt when added to wort; under 0.20 g/kg yeast when added to yeast slurry) increased the number of yeast cells during the middle and late stages of fermentation and also resulted in improved extract consumption. To obtain the desired effect, tannic acid is added to wort before or after pitching yeast (hot wort, cold wort) or can be directly added to yeast slurry. The greater the amount of tannic acid coming in contact with yeast cells, the greater the improvement in fermentation. On the other hand, excessive amounts of tannic acid (more than 4.0 g/kg malt when added to wort, or more than 0.2 g/kg yeast when added to yeast slurry) indicated a negative effect. Therefore, to obtain the desired improvement in fermentation, it is important to properly control the amount of tannic acid introduced, and the timing of its introduction. Improved fermentation was observed for tannic acids in general (hydrolysable tannin and condensed tannin), of which gallotannin, tara tannin, gallic acid (hydrolysable tannin) and persimmon tannin demonstrated particularly prominent effects. The degree of improvement differed according to the tannic acid used. Our data suggest that longstanding problems caused by PYF derived from PYF-malt can be addressed by using certain tannic acids.

O-51

Continuing investigations on malt causing premature yeast flocculation

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Premature yeast flocculation (PYF) is a recurring problem in breweries worldwide. There are many negative fermentation effects attributed to PYF factors, which ultimately lead to beers of low or unacceptable quality. However, due to its sporadic nature, much needed research concerning PYF (with the exception of Japanese and South African researchers) has either not been undertaken or has remained proprietary. Consequently, many questions still abound with regard to the causes and mechanisms of PYF. It is suspected that PYF is induced by compound(s) originating in the malt, surviving through the brewing process and interacting with brewing yeast, resulting in their early removal from the fermenting medium. The nature of this compound (or compounds) is still debated, and many different factors (such as arabinoxylan, β-glucan and ferulic acid) have been described in the literature as being PYF inducers. Previously, we have presented (ASBC Annual Meeting, Victoria, BC, 2007) experimental data for a series of filtrations of wort mashed with PYF-positive malt. The PYF wort was filtered through both a 0.45 μm membrane and a 100 kDa membrane prior to fermentation with a small volume (15 mL) test tube fermentation. It was found that filtration of PYF wort through a 100 kDa membrane reduced PYF activity (as evidenced by absorbance and Plato measurements) compared with the 0.45 μm filtered and unfiltered PYF wort. In continuation of this research, retentate from the 100 kDa PYF wort filtration was collected and inoculated back into 'control' wort for analysis via small volume test tube fermentations. It was confirmed that PYF was induced in an otherwise normal wort through the addition of the 100 kDa PYF retentate. Conversely, retentate (100 kDa) from 'normal' fermenting wort did not induce PYF when reintroduced to a 'control' wort prior to fermentation. In order to determine potential active components of the 100 kDA retentate several pure suspect compounds were added to 'control' wort and fermented. The addition of pure arabinoxylan (medium molecular weight) did not induce PYF. Additions of ferulic acid and β-glucan (medium molecular weight) had variable influence upon addition to 'control' wort. We will report on the screening of this isolated factor and tests conducted to determine the nature of the active component(s) in the 100 kDa retentate.

O-52

Two new technologies for efficient and flexible wort boiling: 1. Rest before wort boiling to convert SMM to DMS; 2. Hop boiling separately from wort

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Abstract not available.

O-53

Procedural operation units during mashing and lautering

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The technological knowledge of the mashing and lautering process is very well analyzed. But, the procedural knowledge of many processes in beer production is still largely unknown, and deeper research was started several years ago. The author's institute at Weihenstephan has worked in this field for many years. Already Herrmann et. al. have tried to increase the procedural knowledge during beer production. The latest results were a new analysis method for particle size characterization during the mashing and lautering process. With this new method, the processes were analyzed. This paper will show the results from former tests based on the latest results which were explored at the institute in recent months. Starting with particle size distribution during the mashing process, I will show the results of experiments using fast motion videos with a miniature mashing container under a microscope. Correlating with this research, analyses with the laser diffraction method will be presented. They show the behavior of various malt qualities and grinding methods on the distribution of the particle sizes and their influences on parameters like β-glucan and gravity. Using the new method, the behavior of particles during the lautering process can also be investigated. They have an important influence on wort flow through the filter cake, whereby a better understanding of the whole lautering process can be found. The aim of the research is to give practical advice for solving problems like changing malt qualities or increasing the brewhouse capacity.

O-54

Comparison of different wort boiling systems and the quality of their worts and resulting beers

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As the brewhouse consumes the major part of energy in the brewery, many efforts have been made to reduce the evaporation rate and therewith the input of energy during the wort boiling process. Brewhouse suppliers have made various suggestions, for instance low pressure wort boiling, thin film evaporation and application of vacuum during boiling or between whirlpool and wort cooler. All of these different systems are actually used by breweries worldwide, however, the quality of the resulting worts and beers are not fully satisfactory in all cases. Furthermore comparison between the different systems has been impossible, as breweries normally have installed only one boiling system in their lines. One of the main goals of the recently introduced "Trial and Research Brewery Weihenstephan" is to have all the facilities with regard to investigations about this part of beer production available in order to find possibilities for improvement. In

order to achieve this, all wort boiling systems were integrated in the brewing line. Beside the conventional system with heating jackets and live steam a wort kettle was installed with both external and internal heat exchangers and a wort pump with a capacity up to 10 circulations of wort volume per hour. The wort running through the external heater can be conducted either directly back into the wort kettle or via an expansion vessel, where evaporation under atmospheric pressure, overpressure or vacuum is possible. All kettles can be operated with a maximum pressure of 3.0 bar abs.—therefore a wide scope of boiling techniques can be performed in this production line. Finally a plant for vacuum evaporation between whirlpool and wort cooler was installed, the wort in the kettle is only kept at boiling temperature without any evaporation. By means of this equipment it was possible, to carry out wide spread research on the influence of the wort boiling system on wort quality under otherwise perfectly identical conditions. In this paper will be given a complete survey of the technical installations and the different possible wort boiling procedures. An overview of the analytical and sensory results of the different worts and beers will complete the presentation.

O-55

Vaporescence versus boiling—Expulsion of aromatic compounds during the whole wort production

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A central function of wort production is the reduction of the content of unwanted aromatic components. Therefore steaming by boiling is a well known separation method that is used worldwide. All available systems have in common that they use vaporization in the form of boiling for the expulsion of unwanted flavors. This work is about the vaporization of wort using vaporescence. The intention is to improve the wort boiling process by prediction of the copper-up-content of aromatic components and to show vaporescence as an undervalued factor influencing the aromatic profile of wort. Both thermo-dynamic procedures of boiling and vaporescence describe the phase-change of a molecule between the vapor and liquid phases, caused by a gradient in the concentration for the reduction of aromatic flavors. In the case of vaporization by boiling the needed energy effects the phase-change in the whole fluid and is identified by steam bubbles. In contrast to that a molecule evaporates by vaporescence on the surface of the liquid phase by withdrawing thermal energy from the environment. The central parameter that has to be used for calculating both steaming-processes is the thermodynamic factor K∞, which describes the equilibrium of the concentration of an aromatic component in the vapor and liquid phases. In the case of boiling only this factor has to be looked for. For the process of vaporescence during mashing, this thermodynamic factor K∞ is extended with a kinetic factor Kg, which is dependent to the diffusive properties of an aromatic component and the Reynolds number of the vapor phase. By researching these factors (K∞, Kg) it is possible to predict the reduction of a flavor like dimethylsulfide by vaporescence in wort production before the boiling process. The understanding of vaporescence of important aromatic compounds allows control of wort production in a new way. Now it is possible to calculate exactly the flavor content in wort production steps like mashing and lautering. Furthermore the combined cognitions of vaporescence and vaporization could be used to describe the residue curve of important aromatic components, like dimethylsulfide, beginning in the mash cooker and ending in the declaration vessel. The results of this work will be used for optimizing the wort production of every brewery. One possibility consists of reducing overall evaporation by predicting the aromatic profile; a second one is given thereby that the processes of mashing and lautering can be seen as new tools for influencing the aromatic profile by using the advantages of temperature dependent thermodynamic factors.

O-56

Isolation of lager yeast mutants with low proteinase A for foam stability of Chinese draft beer

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The foam stability of draft beer is a critical character that reflects beer quality. Although there are many factors that positively and negatively influence foam stability, the most important negative factor is proteinase A. Brewing yeast excretes proteinase A into the fermenting wort during fermentation. Proteinase A diminishes the hydrophobicity of foam-positive polypeptides and reduces beer foam stability. The study mainly focuses on breeding brewing yeast with a low ability to excrete proteinase A during wort fermentation by way of a mutagenic agent, including both nitrosoguanidine (NTG) and ethyl methanesulfonate (EMS). Compared with that of the parent yeast, the results showed a more than 30% decline in protenase A with the mutant yeast at the end of 100 L of pilot fermentation. It opens up the possibility of producing draft beer with foam stability by utilizing mutant strains in future.

O-57

Elimination of diacetyl production in brewer's yeast by relocation of the *ILV2* gene

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Diacetyl has a strong butter-like taste and its presence in lager beer is undesirable. It is produced during fermentation by a non-enzymatic decarboxylation of α-acetolactate in the media. During the maturation period the yeast takes up diacetyl and reduces it to the less flavor-active acetoin. α-Acetolactate (an intermediate of valine biosynthesis) is derived from pyruvate by the action of the *ILV2* gene product. The enzymes involved in valine biosynthesis are expressed in the nucleus with a targeting sequence that directs the gene product to the mitochondria, where valine biosynthesis takes place. Thus far, it has been believed that α-acetolactate formed in the mitochondria is responsible for the diacetyl production observed during fermentation. However, we hypothesized that the precursor of diacetyl might be produced in the cytoplasm. This hypothesis is based on the assumption that Ilv2p in its pro-form is active in the cytoplasm during transport to the mitochondria. In the cytoplasm, the pro-Ilv2p is believed to convert pyruvate to α-acetolactate, which diffuses out of the cell and subsequently is converted to diacetyl. It was speculated that this effect could be circumvented by deleting the nuclear *ILV2* gene and simultaneously, expressing the *ILV2* gene in the mitochondria, thus preventing protein transport through the cytoplasm. A modified $ILV2^m$ gene was constructed by changing the DNA codons using *in vitro* genetic modification techniques allowing for expression in the mitochondria. The resulting $ILV2^m$ gene was inserted into the mitochondrial genome of a laboratory *ilv2* yeast strain using biolistic bombardment techniques and selection for valine prototrophy. Cultivation of the transformants in YPD media showed that diacetyl production was eliminated. Hence, having demonstrated the ability to abolish diacetyl production in a laboratory tester strain, the modified $ILV2^m$ gene was inserted into the mitochondrial genome of a *Saccharomyces pastorianus (Dilv2/Dilv2)* spore clone. The resulting transformants regained their ability to grow without supply of valine. Upon mating, hybrids expressing the modified $ILV2^m$ gene in the mitochondria were selected, all having a valine prototrophic phenotype. Two hybrids were subjected to fermentation trials, and diacetyl production was monitored daily. Both hybrids produced very little diacetyl compared to wild type *S. pastorianus* lager yeast. The results of this study are consistent with the precursor of diacetyl (α-acetolactate) being produced in the cytoplasm in contrast to previous theory. This result may lead to future savings in beer production.

O-58

Gene expression analysis of lager brewing yeast during propagation process using newly developed DNA microarray

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During propagation and fermentation processes in brewing, problems derived from yeast behavior sometimes happen, and so many efforts have been made to solve them. However, it is quite difficult to figure out the critical factors that cause them because yeast metabolism is often considered a sort of "black box". We have completed the whole genome sequence analysis of a representative brewing yeast, *Saccharomyces pastorianus* Weihenstephan Nr. 34. It enabled us to confirm a complicated chromosomal structure in detail and to find the genes specific to lager brewing yeast. Furthermore, a newly developed DNA microarray (LBYG

array; based on lager brewing yeast genome) provided comprehensive analysis of gene expression and offered some explanations of characteristic sulfite production during fermentation (Nakao, Y., *et al.,* Proc. Eur. Brew. Conv., Venice, 2007, 14 pp.). Here we show ability of microarray analysis for practical brewing processes. "Elongation" is observed as yeast morphological changes to pseudohyphal form during yeast propagation or the fermentation process. When it occurs, yeast growth rate tends to decline, and it requires a longer fermentation time that can deteriorate beer quality. In this study, gene expression analysis using the DNA microarray was carried out during propagation to reveal the mechanism of "elongation" at the molecular level and finally establish the optimum condition. Among various brewing factors, aeration was proved to be the most important factor in "elongation", so both normal and elongated yeasts obtained from different aeration conditions in propagation were subjected to gene expression analysis. The resultant gene expression profiles were compared with each other in each biological pathway according to SGD (Saccharomyces Genome Database, Stanford Univ.). Consequently, elongated yeast showed significantly lower gene expression in ergosterol biosynthesis. The number of elongated yeast during propagation was dramatically decreased by addition of ergosterol itself and its intermediate, mevaloate and pantothenate, which is a coenzyme for rate-limiting reaction of this pathway, which supports a hypothesis that lack of ergosterol was the trigger of elongation.

O-59

The role of the yeast vacuole during fermentation

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The vacuole of brewing yeast can occupy as much as 25% of the total intracellular volume. The vacuolar cytoplasm (lumen) is bounded by a membrane (tonoplast), but the structure formed is a totally dynamic organelle with a tendency to coalesce and fragment in response to both environmental stimuli and the physiological status of the cell. One of the reasons for this dynamism is the numerous roles this organelle plays, including maintaining pH and ion status; macromolecule degradation and salvage; protein turnover; osmoregulation; volume regulation; the storage of amino acids, carboxylic acids, carbohydrates and vitamins; and the sequestration of toxins. The morphological changes that occur during pitching and fermentation will be demonstrated. In an attempt to elucidate the rationale for this dynamism during fermentation, this presentation will focus on specific functions of the organelle. Recently the proteins associated with the vacuolar lumen have been identified in laboratory strains using proteomics technologies. Using this as a guide, the expression of the genes that encode these proteins during laboratory and full scale lager fermentations will be discussed in the context of fermentation progression and performance.

O-60

The cleanability of surfaces

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Each year companies in the food and pharma industries suffer high economic losses because of insufficient cleaning, which results in contamination or carry-over. The situation becomes very dramatic if ingredients which can cause allergies get into the product. If there were surfaces with easy-to-clean properties, the whole cleaning procedure would require less time or fewer detergents which provides ecologic and economic advantages. This work investigates the cleanability of surfaces that are relevant for the food industry. Due to the fact that not just the surface of the production plant determines the cleaning success, but also the cleaning media and the contamination itself, a lot of parameters have to be faced. Nevertheless the main focus is on the influence of surface parameters on the detachment of different contaminations, with surface energy, roughness and roughness structure as the topometric and topographic parameters of interest. Cleanability is qualified by measuring the detachment forces of particles on different surfaces. As one of the most critical soilings exists as soon as particles, cells or microorganisms "cooperate" during adhering and attaching to a surface it is extremely important for the cleaning process to investigate the detachment of soilings and biofilms. These experiments were quantified by the measurement of the residue area. The effects of variations in cleaning fluid on detachment also were analyzed. Measurements taken by means of a flow channel and AFM gave very interesting results for the effect of surface quality on its cleanability, as well as new approaches for ongoing research.

O-61

Loop tuning techniques and strategies for the brewing industry

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As today's breweries become more and more automated, the control strategies used to maintain brewery operations become increasingly complicated. Oftentimes, the control strategies employed disregard the impact of upstream and/or downstream systems. When developing a control strategy it is important to take a 'holistic' approach by determining the potential system-wide impact and interaction of individual control loops. This presentation provides a brief introduction to challenges and considerations related to the design and optimization of a modern brewery's PID control systems. This paper explores the relationship between control strategies and their impact on in-line instrumentation, equipment, consistent product quality, hydraulic properties and overall process control objectives. Techniques for analyzing controller performance, identifying interacting behavior, and isolating the root-cause of malfunctioning instrumentation are discussed with several illustrative case studies. The case studies presented in this paper will include examples that demonstrate the impact of pressure spikes, hydraulic hammering, clean-in-place systems and improper design and usage of modulating valves and surge tanks. This paper will also cover basic techniques for tuning proportional-integral-derivative controllers found in a typical brewery (regardless of size). The techniques presented are both simple and powerful, and they allow production staff to set the responsiveness of the controller based upon the process' unique design objective. The approaches covered apply to nearly all types of process control scenarios found at a typical brewery, including temperature, flow and ingredient ratio injection, pressure and level.

O-62

Bioconversion of brewer's spent grains to bioethanol

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Spent grains (SG), the solid cereal residues remaining after extraction of wort, represent a major by-product of brewing and distilling industries. This lignocelluose-rich biomass may provide a source of sugars for fuel ethanol fermentations and may therefore offer potentially valuable alternatives to current uses of SG as animal feedstock (Walker and White, 2007). Bioethanol represents a renewable source of energy (as opposed to synthetic ethanol obtained from crude oils), and it can replace petroleum or can be used as an additive in car engines to increase fuel combustion, octane number and to reduce the emissions of toxic and greenhouse gases. This presentation will review the challenges and opportunities regarding bioconversion of brewer's and distiller's spent grains to biofuels and will also discuss recent results on brewery SG hydrolysis and fermentation to bioethanol. Dilute acid and enzyme treatments were developed to convert the hemicellulose and cellulose fractions of SG from an ale production process to glucose, xylose and arabinose. Pre-treatment of dried, hammer-milled grains with 0.16 N HNO_3 at 121°C for 15 min was chosen as the most suitable method for solubilizing grains prior to enzymatic digestion with cellulase and hemicellulase preparations. Solid loading concentrations (10, 15 and 20% w/v) were compared and reducing sugar concentrations between 40 and 48 g (100 g SG)$^{-1}$ were extracted. Hydrolysate, prepared from 20% SG, pre-treated with 0.16 N HNO_3, partially neutralized to pH 5–6 and digested with enzymes for 18 h, contained 27 g L^{-1} glucose, 16.7 g L^{-1} xylose and 11.9 g L^{-1} arabinose. Fermentation of this hydrolysate for 48 h by *Pichia stipitis* and *Kluyveromyces marxianus* resulted in ethanol conversion yields of 0.25 and 0.18 g ethanol (g sugar)$^{-1}$, respectively. These non-*Saccharomyces* yeasts can ferment C5 sugars, unlike brewing yeast. The fermentation yields, however, were less when compared with fermentation performance on glucose/xylose mixtures in synthetic media, suggesting that inhibitory compounds (possibly furfural) de-

rived from SG were present in the hydrolysate. Our research findings have revealed relatively straightforward chemical and biotechnological approaches to convert brewery and distillery spent grains to bioethanol. "Second generation" bioethanol derived from biowaste material such as SG represents one of the most interesting biofuel sources. Several future challenges remain, however, regarding cost-efficiencies and energy balances of such processes and they will be discussed in this presentation.
References: Walker, GM and White, JS (2007) *Fuelling the future. The science behind fuel alcohol yeast fermentations.* The Brewer & Distiller International 6: 23-27.

O-63

A financial and engineering analysis of energy conservation strategies with respect to heat generation processes within the brewing industry

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As the world moves into the era of high fossil fuel costs and the carbon economy dictates minimization of a company's carbon footprint the need to reutilize valuable energy will become more and more important. So far most breweries have under-utilized the opportunities that their current plant and processes provide for energy recycling. The most common energy recovery process to date has been the wort cooling process used to heat hot liquor. The cooling of wort is a brewing process, and the subsequent energy recovery is a by-product of this process rather then a purpose of the original activity. The purpose of this presentation is to explore energy recovery techniques that may be applicable to the brewing process and to provide an executive analysis of the engineering fundamentals in light of financial limitations and expected returns. The presentation will include examples of current technologies available, a commentary on their potential applications and a critique of their potential limitations. As breweries expand into developing countries and existing breweries modify processes to meet their new operating environments the opportunities to incorporate these technologies into current processes will generate results that have an immediate impact on the financial bottom line. With the move toward triple bottom line reporting such innovations will also provide positive results from an environmental standpoint and allow the company to report on non-financial aspects of its operation. It is my intention to discuss technologies that will be applicable to the boiler, general brewing process, refrigeration, bottling and CIP activates. Examples will be provided as to how such activities are currently being conducted in both breweries and other processing plants. I will also provide a decision-making framework that can be utilized by conference attendees for assessing the benefits of proposed energy recovery projects and the prioritizing of their implementation.

Poster Presentations

P-64

Barley and malt varietal identification using microfluidic lab-on-a-chip technology and automated pattern-matching

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It is important for the maltster to be able identify barley varieties at intake to ensure that the contracted variety is being delivered, it is stored in the correct location and used for production of malt appropriate to its particular attributes or customer demand. Traditional methods for varietal identification of malting barley involve a visual inspection by a skilled operative at point of intake or lengthy analysis post-intake by acid-PAGE (polyacrylamide gel electrophoresis) conducted by highly skilled laboratory personnel. Lab-on-a-chip technology presents an opportunity for the maltster to conduct an alternative method of authenticity analysis at intake. Complete analysis of 10 barley samples and standards can be conducted in around 50 minutes, at a cost of about £1.40 GBP ($2.75 USD) per sample, based on present kit costs. This electrophoresis method is considerably easier, quicker, cheaper and safer than other laboratory-based alternatives. Here, total barley proteins were extracted from a selection of winter and spring varieties and their corresponding malts. Proteins were separated using the Agilent Bioanalyzer 2100/LabChip protein assay system, and the resultant patterns were aligned for comparison using the Nonlinear Dynamics Totallab TL120 DM computerized pattern-recognition software. The suitability of this technology for barley and malt varietal identification and its performance in comparison to acid-PAGE is discussed.

P-65

Near infrared spectroscopy—A useful tool for industrial breweries

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A modern industrial brewery needs tools to keep on top of the standard quality of incoming raw materials as well as to check and control all steps in production process. The cost of everyday analyses has increased, and application of many analyses in laboratory has become almost impossible due to time consumption and high costs. Near infrared spectroscopy (NIR) offers a quick solution in many ways, especially in malt and hops analysis and fermentation process control. NIR spectrometry with Fourier transformation (FT-NIR) has been used, and models for prediction have been developed, validated and used daily for needed analytic characteristics of malt, hops and fermenting wort. The FT-NIR spectrometer Bruker multipurpose analyzer (MPA) was used for all described experiments. Spectra of solid malt samples were taken in reflectance mode. Malt coming into the brewery from industrial malting plants collected within a year was analyzed according to EBC Analytica and MEBAK. The same procedure was repeated with malt produced in micro-malting plant from samples of barley, which served for monitoring of two following barley crops in the Czech Republic. These methods were determined to be references, and results obtained were used for calibration of the NIR spectrometer using OPUS 5.5 software (Bruker, USA). Ground natural hop sample spectra were also measured in reflectance mode, and the NIR system was calibrated for moisture and bitter acids content. Samples of fermenting hopped wort in cylindro-conical vessels (CCV) were analyzed in transmission mode and for calibration used SCABA 5600 (Tecator, Sweden) analysis results. Good correlation between infrared spectra of malt and its very important characteristics based on sugars and proteins and on the degree of their degradation were obtained. Models were able to predict important characteristics of malt such as extract dry, protein dry, soluble nitrogen, Kolbach number and relative extract 45°C with satisfactory accuracy. Application for basic natural hops analysis was also successful, very good results were obtained for moisture, conductometric value, α- and β-acids content. FT-NIR spectroscopy also could be used for quick monitoring of the main fermentation process in CCV; high correlation with spectra was found for alcohol, apparent extract, apparent attenuation and original extract. Models were maintained within two years of usage. This approach is new compared with current published knowledge. The improvement procedure was optimized and systematically and periodically applied to assure the robustness of the methods in spite of changes in the matrix of samples. Rapidity, low time requirements, many analytic results derived from one spectrum and no need of sample preparation are the main advantages of this instrumental method. High instrument costs and the need for calibration based on reference methods are the main disadvantages. FT-NIR may be recommended for beer production at industrial scale, where quality and process monitoring is needed at the moment of production, and the amount of sample needed is high.

P-66

A more efficient and cost-effective method for combined assay of diastatic power enzymes to facilitate routine malt quality evaluation

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An improved, combined extraction protocol in an 8x12 micro well format has been devised that satisfactorily estimates the levels of β-amylase, α-amylase and limit dextrinase in malt. These measurements are achieved with a protocol that is sufficiently labor and substrate cost efficient to enable routine assay to be undertaken by malting quality evaluation laboratories, including breeding programs, maltsters and grain traders. The manuscript for this potential poster presentation was submitted for publication to the *Journal of the American Society of Brewing Chemists* in December 2007.

P-67

bsrA, a genetic marker for beer-spoilage ability of _Pediococcus_ isolates

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The most problematic beer-spoilage bacteria belong to the Gram-positive genera *Lactobacillus* and *Pediococcus*. We have recently shown that while the *horA* gene is highly accurate in determining the beer-spoilage potential for lactobacilli isolates, it is not as accurate at predicting the beer-spoilage ability of pediococci. As such, our goal was to identify genetic markers that could be used for accurate detection of beer-spoilage associated *Pediococcus* isolates. *Pediococcus* isolates that were negative for the putative beer-spoilage associated genes *hitA*, *horA*, *horC* and *ORF5,* yet capable of growing in beer, underwent screening using degenerate PCR primers designed to the ATP-binding cassette region of multidrug resistance (ABCMDR) genes. Resultant amplicons were sequenced to reveal possible identities and functions. Using this approach, we identified several novel ABCMDR genes, one of which has now been highly correlated with the beer-spoilage ability of *Pediococcus* isolates. We have named the novel gene involved *bsrA* (beer-spoilage related). The *bsrA* gene was sequenced by bubble-PCR (genome walking), and it was found that the protein encoded by *bsrA* shares little homology with known proteins but does contain conserved motifs typical of ABCMDR-type proteins. Specific PCR primers were designed to *bsrA*, and used to screen 85 *Lactobacillus* and 50 *Pediococcus* isolates pre- and post-growth in beer. The *bsrA* gene was found in the species *Pediococcus acidilactici*, *Pediococcus claussenii* and *Pediococcus parvulus* and only in isolates capable of growing in beer. Moreover, *bsrA* strongly correlates with resistance of *Pediococcus* isolates to hop-compounds (*t*-test, $P < 0.005$). Interestingly, *bsrA* was not found in any *Lactobacillus* isolates, whether able to grow in beer or not. This is the first gene that we are aware of that differentiates between lactobacilli and pediococci that are able to grow in beer. It also should be noted that using *bsrA* as a marker for determining the beer-spoilage ability of *Pediococcus* strains is an improvement over using only *horA* in this regard (accuracy of 89–90% versus 78–80%, respectively). In fact, by combining *bsrA* and *horA* in a multiplex PCR, an even better accuracy of 92–94% can be attained for the prediction of the spoilage potential of contaminating *Pediococcus* isolates. Since detection of *horA* has an 85–88% accuracy for prediction of beer-spoilage potential of *Lactobacillus* isolates, a *bsrA/horA* multiplex PCR is the best currently available approach for assessing the beer-spoilage potential of both lactobacilli and pediococci found in breweries.

P-68

Mycotoxin lateral flow assays—A new approach for mycotoxin analysis

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Mycotoxins are secondary metabolites formed by fungi. Diseases, which are caused by an intake of mycotoxins, are called mycotoxicoses. For humans and animals two kinds of fungi are of most importance concerning the contamination of food and feed. *Fusarium* species, belonging to the group of field fungi, are generated directly on agricultural crops. The respective mycotoxins, e.g. deoxynivalenol (DON), predominantly reach the food chain through cereals and cereal products. The other important group of fungi (e.g. *Aspergillus* and *Penicillium* species) occurs during inadequate storage conditions. The corresponding mycotoxins, e.g. aflatoxins, are found in incorrectly stored crop products or food products. Deoxynivalenol is a low molecular weight metabolite of the tricothecene mycotoxin group produced by fungi of the *Fusarium* genus, particularly *F. graminearum*. These fungi occur widely and will infect barley, wheat, and corn. Deoxynivalenol, or also called vomitoxin, is highly toxic, producing a wide range of immunological disturbances. The maximum residue limits or other international regulations for some mycotoxins requires appropriate analytical methods. The RIDA®QUICK Mycotoxin product line is a novel immunochromatographic lateral flow format for the detection of aflatoxin, and deoxynivalenol from grain and cereals. The inverted competitive assay format is based on the directly proportional reaction of the target molecule with specific gold-labeled antibodies. This means as soon as mycotoxins above detection level are presented, a result line occurs. A control line assures the validity of the test run. After extraction and sedimentation the sample is applied to the test membrane. Depending on incubation time, between 2 and 16 minutes, different concentrations of applied mycotoxins can be determined semi-quantitatively visually. For documentation purposes the reaction can be stopped and the sticks can be stored for several months. An alternative to the visual detection, a quantitative evaluation of the intensity of the colored line results, is possible using the new RIDA®QUICK lateral flow reader. This portable scanner allows objective optical detection and interpretation of results. The data can be exported and saved for documentation. Increasing demands on quality control and consumer protection require more and more fast and reliable testing of raw materials and processed foods. Contemporary assessment of raw materials guarantee cost- and time-efficient distribution and Production. The new RIDA®QUICK DON and RIDA®QUICK aflatoxin, optional combined with the RIDA®QUICK SCAN, fulfills the requirements of modern mycotoxin analysis.

P-69

RIDASCREEN® gliadin competitive assay—Gluten analysis in hydrolyzed food samples like beer, starches and syrups

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Celiac disease is becoming a major gastrointestinal disease, and it is increasingly in the focus of scientific discussions. It is a permanent inflammatory disease of the upper small intestine in genetically susceptible individuals induced by ingestion of storage proteins from wheat (gluten), rye and barley. The classic picture is poor growth, weight loss, diarrhea and increased fat excretion in stool. Celiac disease is currently considered to be an autoimmune disease and shows different severe site effects like osteopenia, neurological disorders, anemia, vitamin deficiency and others. In the US a prevalence of 1 in 133 persons was published. Much higher prevalence, 1 in 300, has been reported for Europe. Some foods contain highly processed cereal proteins, e.g. beer, starches or syrups. Hydrolyzed proteins do not allow the use of classic sandwich ELISA methods for determination of gliadins. R-Biopharm has developed a competitive enzyme immunoassay for the detection of gliadin from those samples, the RIDASCREEN® Gliadin competitive assay. Generally prolamins in food or food ingredients can be hydrolyzed or partially hydrolyzed during processing or can occur in food through their use as functional ingredients. During proteolytic treatments, prolamins are partially hydrolyzed in more or less large fragments containing two or more epitopes and in small fragments having only one epitope or motif. Consequently, small hydrolyzed fragments with a unique epitope cannot be reliable determined by a sandwich ELISA. Actually it has not been verified which amounts of smaller fragments are still toxic for celiacs. Further clinical studies are required. A 33 amino acid peptide from gliadin that is resistant to gastric and pancreatic hydrolysis and acts as a strong stimulator to intestinal T cells is thought to be the toxic sequence. Sub-sequences of this peptide were used to check for their reactivity with the R5 antibody. This antibody is internationally recognized as the most fitting for determination of the gliadin content in foodstuffs. A small peptide, QQPFP, was selected for the development of the competitive gliadin ELISA. In-house studies using hydrolyzed starches, syrups and beer showed in many cases notably higher "gliadin concentrations", compared to analysis using the classic sandwich ELISA. The ELISA detects the intact molecule as well as fragments down to one epitope. The RIDASCREEN® Gliadin competitive assay offers additional information when testing beer, syrup or starch samples. RIDASCREEN® Gliadin (sandwich ELISA) negative samples can be tested again to see whether smaller potentially toxic gliadin fragments are present. This limits the risk for celiac patients and allows industries to confirm their naturally gluten-free labeling.

P-70

Identification of novel foam-related proteins through two-dimensional gel electrophoresis analysis of the beer proteins

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Foam stability is one of the important characteristics in beer brewing. The purpose of this study was to identify foam-related proteins using two-dimensional gel electrophoresis (2DE) analysis of the beer proteins. We brewed a total of 25 beer samples, each brewed from a malt with different modification in one of the three cultivars (cultivars A, B and C). In cultivar A, the foam stability did not change even when malt modification increased. However, the foam stability decreased in cultivars B and C with increased modification. To investigate foam-related proteins, we collected beer proteins in three fractions, namely beer whole proteins, salt-precipitated proteins and the proteins concentrated from beer foam. 2DE analysis of these protein fractions revealed that protein spot b2 in cultivar A did not change in any of the three protein fractions even when the malt modification increased, although spot b2 in both cultivars B and C decreased. Pre-spot intensity, each spot intensity against all spots, was calculated by vol. % as a unit. The spot intensity was calculated by multiplying the beer protein concentration and the pre-spot intensity by dimensionless as a unit. The foam stability of 25 beer samples significantly correlated with the intensity of spot b2 at the 5% level ($r = 0.503$). Furthermore, we focused on other 2 major protein spots (b0 and b5) observed in 2DE gels in all-malt beer samples with different foam stability. Subsequently, multiple regression was analyzed on 25 beer samples by the spot intensities of spots b0, b2 and b5. As a result, 72.1% of the variation in beer foam stability was explained by the intensities of spots b0 and b2 as positive, and spot b5 as negative explanatory variables. Similarly, 85.6% of the variation in beer foam stability of 10 commercially available beer samples was explained by the intensities of spots b0 and b2 as positive and the spot b5 as negative explanatory variables. MALDI TOF-MS and LC-MS/MS analyses followed by database search revealed that the protein spots b0, b2 and b5 were identified as protein Z originating from barley, barley dimeric α-amylase inhibitor I (BDAI-I) and thioredoxin originating from yeast, respectively. These results suggest that BDAI-I and protein Z are foam-positive proteins (or indicators), and yeast thioredoxin is a foam-negative protein (or indicator). We identified BDAI-I and yeast thioredoxin as novel foam-related proteins.

P-71

The Determination of intact acetolactate concentrations in fermented products without prior conversion to diacetyl or acetoin

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Diacetyl is a key flavor compound in fermented foods and beverages. It is not formed directly by bacterial or yeast metabolism, but is formed by the spontaneous conversion of acetolactate produced by microorganisms during fermentation. Therefore, in order to control the concentration of diacetyl in the finished product, it is necessary not only to measure the concentration of diacetyl itself, but also that of its precursor acetolactate. As acetolactate is a highly labile compound, it is usually measured after being converted to diacetyl. However, this method of analysis has two major shortcomings; 1) the percentage conversion is not always consistent, and 2) it has a relatively long conversion time (a 90 min reaction time is recommended in the official BCOJ analytical method). In order to eliminate these obstacles, the authors have developed a method of measuring intact acetolactate without the need to convert the compound into diacetyl or acetoin. The method utilizes the measurement of NADPH oxidation brought about by the conversion of acetolactate into dihydroxyisovalerate by the enzyme acetolactate reductoisomerase. We produced a recombinant *Aspergillus oryzae* harboring the acetolactate reductoisomerase of *A. oryzae* (AoIlvC) on an over-expression plasmid, and purified the recombinant AoIlvC from the soluble cytoplasmic fraction. This assay method was not influenced by the presence of high concentrations of diacetyl or acetoin. This method of analysis makes it possible to measure the concentrations of acetolactate in fermenting mash during *sake* (rice wine) brewing and in milk products produced by fermentation with lactic acid bacteria.

P-72

Evaluation of optical O_2 measurement compared with the electro-chemical O_2 measuring system

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The determination of O_2 concentration plays an important role in the different stages of beer production. The research center at Weihenstephan (Technical University Munich) investigated the new optical O_2 measurement and compared it with the traditional electro-chemical O_2 measuring system. Special attention was paid to the testing of the optical O_2 measurement's accuracy and precision. The results were compared with the results of accredited electro-chemical O_2 measurement in five different tests. The paper will present information on the test facility and procedure, discuss the test results, and present a conclusion that confirms that the optical O_2 measurement is well suited for the determination of O_2 content in the brewing and soft drinks industries. Compared to the electro-chemical O_2 measurement, the optical O_2 measurement provides faster response times and does not require frequent calibration.

P-73

Matrix foaming potential—A useful tool for foamability prediction

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There are quality parameters that can be easily evaluated by any customer and that should be crucial for a brewmaster to take care about. Foamability is one of them. As with all other quality parameters, brewers need a tool to measure foamability under standard conditions and have values concerning their foam as soon as possible. It is best to know foamability even before a beer is finished. This can be done by measuring its matrix foaming potential (MFP). The matrix for these measurements can be all the intermediate products in a brewery production line. These measurements come from the idea that the foaming potential of beer is hidden in its raw materials and process management. Measurement of MFP is then a useful tool for process control and optimization. Another task for MFP measurements is searching for foam-destructive operations over the production line and eliminating them. For MFP measurements a foamability meter (1-CUBE, Czech Republic) is used. A sample of sweet wort, hopped wort, fermenting wort, green beer or beer is degassed (if necessary), stirred with an injection of foaming gas (air, nitrogen, carbon dioxide or mixture of these), and standardized foam is generated. The height of the head is then measured in time, and the time needed to reach a given height is recorded. MFP results from beer measurements show good correlation with other foam stability measurements (NIBEM) and with foam behavior in a customer's glass. MFP values from intermediate products also show satisfactory correlation with the foaming potential of finished beer.

P-74

Optimized analytical methods for the determination of SO_2 in beer and malt

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The determination of the SO_2 content in beer is becoming more of a focus for brewers. The technological importance of SO_2 is based on its antioxidative potential, which protects beer against oxidation and therefore enhances flavor stability. However SO_2 is said to have some allergenic potential, especially for sensitive individuals it may cause hives, stomach problems and a headache, even though the content in beer is considered to be harmless from a physiological point of view. Nevertheless the allergenic potential has provoked a European directive, which is limiting the content of SO_2 and sulfites to a concentration of 10 mg/L, otherwise it has to be labeled. Based on these backgrounds, different analytical methods for the determination of SO_2 were compared among each other according to their accuracy, application, linearity, precision and selectivity and have been optimized. The oldest and most used analysis in brewing is the distillation method according to Monnier-Williams. Due to the high expenditure of time, personnel costs and a recovery rate of ≈90% this analysis is

less suitable for determination in beer. Opposite to that for determination in malt it is more appropriate, because there is no adequate possibility of extracting the SO_2 from the malt without higher losses. Comparative measurements using whole malt kernels show significantly higher yields with much lower coefficients of variation compared to fine and coarse grist. The reasons are the oxidation of the SO_2 during the milling process and mainly the fact that in the flask agglutination occurs by fine and coarse grist in contrast to whole malt kernels. It is generally possible to determine the SO_2 content in whole malt kernels, because of the fact that the SO_2 is primarily located in the husks. The suitability of the new method could be confirmed in several interlaboratory tests. Photometric analysis via CFA (continuous flow analysis), using pararosaniline, is another possible method. This method showed significantly higher results for SO_2 in beer compared to the distillation method. These higher results can be explained by the occurrence of background signals caused by the beer matrix. It could be demonstrated that the background signals are mainly caused by Maillard reaction products in the beer. By using new developed Teflon membranes it was possible to eliminate the interfering signals almost completely and led to a significant increase in the sensitivity of this method. In recent years, ion chromatography (IC) has been established more and more in breweries for the determination of SO_2. IC is characterized by a low standard deviation, good reproducibility of the individual values and a good recovery of up to 100%.

P-75

Brewing industry quality control applications using headspace sampling/gas chromatography

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There are four major HS/GC analyses that are performed at breweries for the purpose of quality control and identifying problems or changes occurring in brewing and fermentation processes that can adversely affect both the taste and quality of the final product. The first and perhaps most important of these is monitoring for vicinal diketones (VDK's) in beer. VDK's are known to affect the taste of beer, imparting a butter-like flavor that is considered undesirable at higher concentrations. Generally speaking, heavier beers, including stouts etc., tend to have higher levels of VDK's than do lighter beers. Typically, VDK's are found in the 1–50 ppb range in lighter beers and can exceed several hundred ppb in darker beers. Acetaldehyde is a further analysis performed throughout the brewing process. Acetaldehyde is reduced to ethanol by yeast during secondary fermentation, but oxidation of the finished beer may reverse this process, converting ethanol back to acetaldehyde. The taste and aroma of acetaldehyde has been described as fresh cut green apples, grass, leaves and even latex paint. Levels of acetaldehyde are generally in the 1–20 ppm range. A third group of compounds of interest are the trihalomethanes. These are usually introduced into the beer through the municipal water supply. Municipal water is often treated with chlorine, resulting in a variety of chlorinated disinfection by-products. Chloroform is usually the most prominent trihalomethane identified during this analysis. The fourth test commonly performed on beer is the isolation and identification of sulfur compounds. Dimethyl sulfide (DMS), sulfur dioxide (SO_2) and hydrogen sulfide (H_2S) are of particular interest to brewers worldwide. DMS has the taste and aroma of sweet corn. This comes either from the malt, as a result of a short boil of the wort, slow wort chilling or bacterial infection. SO_2 is often used as a preservative. Various types of yeast will produce significantly different levels of H_2S. Above very low ppb levels, these sulfur compounds give off an unpleasant taste and smell (e.g., rotten eggs). Though traditionally these four QC tests have been performed individually, the use of the PerkinElmer HS/GC allows for the combining of these analyses, thus dramatically enhancing productivity. In fact, depending on one's desired level of detection, all four methods can be analyzed simultaneously or, if need be, broken up into method specific parameters. The improved application will be described and criteria for performance and quality control will be outlined.

P-76

Quantitatively identifying PYF malt: Statistical modeling of yeast in suspension in small scale fermentations

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When analyzing small scale and tall tube fermentations, yeast in suspension was conventionally estimated by taking spectrophotometric measurements (600–800 nm) at specific time intervals. The collected data was then presented graphically and examined visually. Often, differences in visual observations between 'good' and PYF malts are the determining factor in identifying problematic malts. Due to natural variation in these pilot or lab-scale fermentations, qualitative identification of PYF malt can be subjective. Secondly, qualitative observations are difficult to translate from lab to lab. In this study, a statistical method of comparing the dependence of yeast in suspension with time is presented. Wort from a known PYF malt and a control malt were fermented in 15 mL test tubes with 4% glucose (w/v) at 21°C (method accepted J. ASBC). The fermentation was analyzed optically every 5.0 min for 72 hr without disturbance using an inexpensive and easily constructed photometer/laser/data logger system. The resulting 864 data points yielded curves that were subsequently modeled using a piecewise regression technique. The continuous absorbance data exhibited different (curvilinear) behavior before and after the maximum absorbance 'breakpoint'. Piecewise regressions were undertaken using the non-linear regression module of the Systat statistics package. The software determines the best fit of two functions, (one before and one after the breakpoint) by minimizing the sum of squares of the regression. We will report on the suitability of various functions (i.e., exponential, Gompertz, normal, logistic and Verhulst) to describe the absorbance data. The modeling technique permitted quantitative and definitive comparisons between PYF and control fermentations by providing the best fit parameters of the two functions, the breakpoint values and related asymptotic standard errors. The qualitative differences between the PYF and control absorbance data obtained by this modeling technique will be presented. The techniques discussed here allow improved criteria to be utilized when identifying a PYF malt. The technique may also add to our ability to track and optimize fermentation performance.

P-77

Comparison of different methods to count yeast cells

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Three different cell counting procedures are compared to determine yeast cell counts in pitching yeast and in young beer. The classic Thoma chamber and microscope were used for direct determination of yeast cells. Furthermore a cell counter (AL-Counter, Al-Systems) which measures cells via electrical resistance in solution and a cell counter (NucleoCounter YC-100, ChemoMetec) were used. The NucleoCounter has the additional ability to determine viable cells by automatic staining. All systems were investigated according to precision, repeatability and standard deviation. Furthermore it was tested to find optimum working conditions regarding cell count to achieve repeatable results of each method. Viable cells could only be determined by the direct counting method with a Thoma chamber and NucleoCounter. For this parameter standard deviation and optimum range of operation were investigated too. To achieve reproducible results three different yeast strains were used for the investigations characterized by more or fewer flocculating properties. The results showed a good reproducibility for AL-Counter and NucleoCounter for different yeast strains. The cell counting by the Thoma chamber had the following advantages. This system has a low investment cost if a microscope is present. Another advantage of the Thoma chamber is the possibility of the visible inspection of the yeast cells, even though it is a time-consuming method and needs a well-trained staff. The precision of results is much lower compared to cell counter methods and will be influenced by several factors. The AL-Counter offers advantages through the automation of cell counting. Furthermore a calibrated dilution step of high concentrated samples is possible. Another advantage is the cell count determination in a high

yeast concentration range. Disadvantages may be the lack of determination of viability and the greater effort in work due to the additional dilution step. Furthermore trub particles may be lead to incorrect results as well as cells in higher concentrations. The NucleoCounter system offers the following advantages. One of the major ones is the ability to determine the viability of yeast cells. Handling of this equipment is very easy, and it is easy to learn. There is also no calibration or maintenance needed. On the other hand the dilution range of samples has to be taken into consideration to achieve precise results.

P-78

Liquid phase primary dissolved oxygen calibration for package analyzers

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GEM-F calibrator systems offer the beverage industry the ideal solution to that elusive target—a practical and reproducible means of presenting both portable and package analyzers with clean liquid samples containing accurate, known DO levels. Because this is performed rapidly and economically using an on-site facility, Headmaster maintains it is a more powerful QA tool than schemes using "reference can" batches, which rely on statistical processing of results from many individual samples. GEM's two series-operated gas/liquid contactors give a stable permeable 2.8 m^2 interface between counter-flowing gas and water. Pressure and flow rates are fixed to deliver a stable >99.99% saturation of outlet water with the inlet gas. This property of the contactor system is ensured by built-in design redundancy and can be confirmed using feed gas which is oxygen-free or has a known oxygen concentration. The output liquid DO level is therefore always defined, using the O_2 solubility algorithm, by outlet water temperature, feed gas pressure, and oxygen concentration in the internally-blended feed gas. Operating at constant pressure and gas concentrations, GEM computes and displays temperature-corrected DO levels using proprietary circuitry. The systems are justifiably described as calibrators because all process parameters can be checked independently with calibrated instruments so that displayed and actual DO values can be compared. DO levels are chosen to suit product applications, recognizing analyzer characteristics and practical tolerances in the calibrator's key parameters (gas O_2 %, temperature, pressure). Typically, 150–300 ppb (5 ppb tolerance) is used for low DO products such as beers and ca. 750 ppb (10 ppb tolerance) for wines. F-format systems include interface units enabling Headmaster's reusable sample bottles (SB) to be pre-purged with the same gas and to the same pressure used in the calibrator's contactors, so filling is at constant DO level and results in a known total package gas level. SB is designed for filling to a head-space of either 10 ml or 1 ml: the latter is useful if QA focuses on package liquid DO and if filling and sampling temperatures are different. Left in air, the bottles are valid calibration samples (+/–5 ppb DO) provided they are sampled within 2 or 3 hours of filling. For situations involving longer delays, O_2 ingress through the closure and connectors is avoided by holding SBs in sealable outer housings purged to 1% O_2. These protected SBs, shipped from another calibrator facility, provide for independent validation of the analyzer and calibrator.

P-79

The oxidative capacity of rosemarinic acid and a catalase/superoxide dismutase mimetic using an adapted europium tetracycline based hydrogen peroxide assay

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We describe the application of a novel fluorescence method to measure hydrogen peroxide levels in beer. The method can be used as a diagnostic tool in predictive beer ageing tests. It can also be used to determine the effects of hop varieties, malt, and emerging processing aids, like rosemary extract, on reactive oxygen suppressive character. It is based on the behavior of europium tetracycline complex (EuTC). EuTC is naturally fluorescent, but when bound to peroxide the fluorescence yield increases by 15 times or more, with a detection limit around 1 micromolar. The method is cheap and reliable and capable of formatting with 96 well plates. It requires a fluorimeter, but these are generally cheaper than the instruments required to perform the luminol-based chemiluminescence peroxide assay. The EuTC-based peroxide assay was used to compare the effects of herbal extracts, extracts from different hop varieties, and fractionated malt extracts on peroxide accumulation during ageing at elevated temperatures. This enabled us to compare the equivalent 'reductive character' of these fractions with respect to sulfite. Trials with rosemary extracts show that the rosemary could replace exogenous sulfite addition without affecting quality profiles for a range of different beer types. Sulfite oxidizes peroxide to water and oxygen, and in the process is converted to sulfate. However the extracts work differently. They possess catalase and superoxide dismutase activity. Classically catalase coverts peroxide to water and oxygen. Superoxide dismutase converts the super-anion to peroxide which in the presence of the catalase is transformed into water and oxygen. Superoxide requires an electron donor, and polyphenols in the beer could provide these reducing substrates. The activity is heat stable, so the activities are not due to classic protein-based enzymology. Small molecules with catalytic properties have been described as mimetics. Molecules with enzyme-like activity are small and usually heat stable. Salens are one such example; they are crescent shaped heterocyclic molecules which can bind a metal ion, often manganese, in the cleft. We were able to show that these molecules have both SOD activity and also catalase activity using the EuTC assay. And, that the activity operates in a beer matrix. In theory salen can break down peroxide indefinitely without any supporting reductive substrate. This is quite unlike the terminal role of sulfite. It acts as a reactant to destroy peroxide. It seems inevitable that developments being reported for anti-oxidant, therapeutic mimetics will spill over into the food and beverage industries. Our results show how these products could manage quality and improve shelf life. The EuTC assay can be used to assay product, improve process quality and guide innovation.

P-80

Beer foam generation and its collapse description

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Foam stability is a very important parameter for beer quality. There are various methods available to measure it, but there is still a requirement for a reliable and quick method of assessing foam quality and stability. There are two ways of pouring or dispensing beer from the bottle or keg. Both foams differ meaningfully having different properties such as amount of foam, size distribution of bubbles, foam appearance, including whiteness, texture and the tendency to adhere to the glass wall (lacing or cling). The stability of foam depends both on the kind of beer and foam formation. Foam generation methods can comprise free fall of beer into glasses, introduction of gas, shaking, mixing or beer passing through the nozzle and powder or electrolytic release of carbon dioxide from the saturated beer. Automated methods for the measurement of beer foam collapse rate have also been described. There are two methods generally used in many laboratories, the NIBEM method and Lg-analyzer measurement. The third method formerly widely used was the Ross Clark determination of foam stability. The new instrument for beer foam stability measurement described in this work was tested, and various models of beer foam decay were assessed. The beer flowed under low (10–20 kPa) or high (200–300 kPa) pressure through a thin tube or nozzle into glass cylinder, and the same beer was manually poured into the beer glass. Foam surface fall was followed over the whole degradation curve. The sensor movement was stopped 0.5 mm above beer level to avoid long time measurement. The foam tester consisted of a needle conductivity sensor controlled by a step motor, which enabled us to follow the foam surface fall during its collapse. The measured data were collected automatically through RS 232 and loaded into a computer. The stirring method was also estimated, during which beer stirring together with gas introduced into beer and beer intermediates was used for foam generation. Quadratic, exponential and two-step consecutive kinetic models were used for foam decay after foam generation in these trials. The first two models provided a precise description for nearly the whole foam degradation curve, but the meaning of the parameters was well defined and understandable only for the first part of the foam collapse. The third method gave a reasonable description for the whole curve. The stirring method describing fast beer collapse must involve both the foam generation and degradation into decay equation. Examples of the beer, production intermediates and model solutions contain-

ing foam-active and foam-destroying compounds are presented and discussed.

P-81

Fluorescence microplate readers as an alternative to flow injection analysis for determination of wort β-glucan

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Selection of new barley varieties to meet the needs of the malting and brewing industry requires that the lines being developed meet a set of malting quality standards that have been developed to help maltsters and brewers predict the commercial performance of the barley and resulting malt. One important parameter of the malting quality determinations is the level of β-glucan found in wort, since high wort β-glucan levels are thought to predict potential problems with brewhouse filtration. Historically, malting quality assessments have been made relatively late in the process of line development, when sufficient grain is available to allow production and use of the 55 grams of malt called for in the standard Congress wort production method (ASBC Malt-4). Recently, a modification of the Malt-4 method producing representative Congress worts with significantly smaller amounts (<200 mg) of malt has been presented (Schmitt et al., JASBC 64, 181–186 (2006)). This adaptation of the standard Congress mashing cycle allows malting quality assessments to be made much earlier in the breeding cycle, improving the efficiency of the malting-grade line selection process. However, the smaller volumes of worts produced with the new mashing protocol were not sufficient for measurement of wort β-glucan by traditional flow-injection analysis systems referred to in Wort-18, the standard ASBC method for determination of β-glucan in Congress wort by fluorescence. However, the chemistry that is the basis for Wort-18 is still appropriate for use in fluorescence detection systems utilizing smaller volumes of samples and reagents, such as commonly available microplate fluorescence readers. In this presentation, we show that simple adjustments in sample volume and similar parameters allow the use of a fluorescence microplate reader as an alternative for determination of β-glucan in Congress wort. In addition to making fluorescent β-glucan analysis feasible for researchers with limited quantities of malt or wort, or those without access to suitable flow injection analysis systems, the microplate format β-glucan analysis procedure allows a significantly greater number of sample treatments to be analyzed. Such increased analytical capacity may enable additional experimental treatments not previously feasible with conventional flow injection systems.

P-82

Examination of the relationships between alcohol, original, real and apparent extracts in pilot plant and commercially produced beers

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Since the time of Balling (1865), brewers have been interested in and reported on the density relationships between wort and fermented beer. Most of these researchers were concerned with the relationships between wort, beer extract and alcohol content and published their work in German over a century ago. Approximations based on relationships of the four brewing parameters, alcohol content, original, apparent and real extracts (i.e., Aw/w OE, AE and RE) were first reported by Balling (1865) and Holzner (1877). These approximations (which were reported to be somewhat influenced by OE) were developed long before linear regression methods were developed in 1896. Aside from these approximations found in the brewing literature (e.g., *Handbook of Brewing*, 1st Edition, 1995), numerous websites report on and provide online calculators to estimate Aw/w, OE, AE or RE knowing two of the four brewing parameters. However, somewhat astoundingly, almost all the reports of these 'approximate' relationships lack any report of the error associated with their calculations. Given the development in brewing sciences and advances in chemical and statistical analyses, one might argue it is long overdue to re-examine the values of the brewing values reported in the 1800s. In this paper we will report various statistical analyses of relationships between Aw/w OE, AE, and RE, as well as ratios between the corrected real and apparent degrees of fermentation (RDF/ADF) using a dataset of brewing parameters for 821 pilot plant and commercial beers. The derivations and dependencies of these relationships on the original extract relation as reported by Balling will also be reported. We will also report on the error of these various fundamental and empirical relationships. New predictions of AE calculated as a function of Aw/w and RE analogous to the improved Tabarie's formula will also be reported. Finally, the suitability of the formula used in Great Britain to calculate alcohol levels using original and final gravity values for excise purposes will be commented upon. We expect this paper will be of use to brewers in order to more accurately estimate Aw/w and real extract values.

P-83

Analysis of trans-2-nonenal in beer using solid-phase micro extraction with on-fiber derivatization and gas chromatography/mass spectrometry

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Flavor stability of beer is still a challenging issue for all brewers. trans-2-Nonenal (T2N) especially is considered to play an important role in the deterioration of beer flavor and aroma during storage. Usually analysis data of T2N has used as an index for freshness of beer. For analysis of T2N in beer, the HPLC method using precolumn derivatization and column switching techniques was reported; however, this method had some problems, which were time-consuming; much use of materials and low reproducibility originated from many isolation steps in this method. In this work, we adopted solid-phase micro extraction (SPME) with on-fiber derivatization and gas chromatography/mass spectrometry (GC/MS) with a new internal standard as a solution for these problems. On-fiber derivatization was conducted using O-(2,3,4,5,6-pentafluorobenzyl)-hydroxylamine (PFBOA), which was absorbed onto a stable flex divinylbenzene/carboxen/polydimethylsiloxane 50/30 μm fiber and exposed to the headspace of a vial with a beer sample. T2N selectively reacted with PFBOA, and the oximes formed were desorbed into a gas chromatograph injection port, detected and quantified by mass spectrometry with (2,3-D2)-trans-2-nonenal as an internal standard, which was detected separately from the target T2N. Because this newly adopted internal standard had the same retention time as the target T2N, it enabled a very stable method for analysis of T2N with high reproducibility. As a result, this method detected T2N to a 0.005 μg/L level, which accordingly showed higher sensitivity compared with the existing method. SPME with on-fiber method and the following GC/MS don't require complex derivatization steps and much solvent, thus adopting this method also enables simple and fast analysis of T2N, which would lead to effective solutions for improving the flavor stability of beer.

P-84

Development of new evaporation technology to improve flavor stability

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Recently, it has been reported that lower thermal stress for the wort improves the flavor stability of beer. As a result of development of modern boiling systems in recent years, sufficient functions can be performed even at shorter boiling times and with lower total evaporation. These new boiling systems are very effective for reducing thermal stress for the wort, but generally they require an enormous investment. In this study, we investigated a new evaporation technology with inert gas that can be installed easily in existent boiling systems. It has been noted that blowing of inert gas to wort can accelerate evaporation of aroma compounds, but the optimum blowing conditions have not been fully studied. We found that DMS was stripped off most efficiently when the boiling process was split into two phases: boiling phase and stripping phase with inert gas blowing. In this case, the improvement tendency for flavor stability was found without any significant influence on fermentation or beer quality. As a result of further research, we found this effect could be obtained in the case of blowing into the piping during transfer from wort kettle to whirlpool and stripping at the whirlpool without the serious problem of hot break. This new evaporation technology is very easy to install in existent boiling sys-

tems and might be attractive especially for breweries which have surplus carbon dioxide or nitrogen.

P-85

Novel solution for the wort boiling process—Low cost enhancement of wort boiling systems

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Both quality of beer and production costs depend on wort treatment performance during the wort boiling process. Wort treatment in systems with an internal evaporator implies flow induced inhomogeneities and thus ineffective wort processing. Basing on fluid mechanical research of the flow inside the wort boiling kettle with an internal evaporator a subjet concept for the low cost optimization of the system was developed. Herein, the deflector of the boiler is placed under the wort surface. Hence, the wort enters the annulus of the kettle horizontally under the liquid surface. The main characteristics of the novel system consist of the elimination of unwanted oscillations of the boiler during heating up and the development of an enhanced, technologically adapted flow in the kettle. In this work, the flow characteristic and physical phenomena that occur during the heating up and boiling stage in standard and subjet system configurations will be compared and presented together with preliminary technological results for subjet boiling. The experimental investigations were carried out in a one tube natural circulation evaporator of industrial scale and a wort kettle of 19 hl volume with a steam heated internal boiler. The flow in the kettle was investigated experimentally by laser Doppler anemometry and numerically by means of CFD. Technological investigations were carried out with 12% extract wort. The heating up process started with a temperature of 65°C, and the boiling process took place at atmospheric pressure for 45 min with an evaporation of 3%. The standard system revealed unsteady behavior during the heating up phase. Severe flow unsteadiness (geysering) led to increased fouling and consequently to shortening of the intercleaning period. This phenomenon was not found in the subjet system. Here, the heating up process was smooth, without flow oscillations. Both numerical and experimental flow investigations during boiling in the conventional system revealed the presence of a significant short circuit flow in the kettle. Short circuit flows lead to non-homogeneous wort treatment and increased energy demand. In contrast, the short circuit did not occur in the subjet system. Herein, increased momentum transport between the free jet and the matrix fluid favored a homogenous flow arrangement. During wort boiling, free DMS content decreased from 133 to 17 µg/l while the TBZ-number increased by only 14 points. The results reveal that the subjet realizes a homogenous, thermally and mechanically gentle treatment of wort. The system is characterized by low evaporation, low energy supply and elevated processing efficiency.

P-86

Lauter tun operation: Practical application of lautering theory

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There are many texts and papers covering the theory of lautering. However, few of these deal with the practical operations of a lauter tun and how to best achieve optimum throughput. Although each vessel is different, this paper will take the theory of lautering and apply it to a practical application in a working brewery and also highlight modifications made to the control and operation of this specific lauter tun. It will cover the basic theory, practical application, and overall operations of the lauter tun, including different practices in lautering and separation procedures that brewers can use in order to optimize their own lauter tun performance.

P-87

XXL mash filters—Technological results from new generation mash filter systems

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Mash filters are gaining importance in the technology of wort separation with growing brewhouse capacities and units. Even though they have been in existence for several decades the unit size was limited to approximately 12 tons of grist charge for many years. High gravity brewing, faster turnaround times and higher utilization of brewing lines made it necessary to develop mash filter units of larger size. The actual size of mash filters is beyond 26 tons of grist. They have now been in operation for several years. This paper describes the differences in construction and process performance compared with previous constructions. Several details lead to improved technical performance. Technologically the new filter generation gives very good results in yield, occupation time and overall capacity. The wort quality compares very well with previous systems. This paper describes technical improvements supported by technological effects. The central rail support system allows fast mechanical movements and a smooth and even mash transfer and distribution. Efficiency provides yields above laboratory values, while solids are low in general, accompanied by good turbidity. All quality parameters were measured during commissioning of new full size filters equipped with this technique. Technical highlights: up to 26 t of malt grist, chamber plates 2.4 x 2.4 m, 14 brews of high quality wort per day.

P-88

Wort boiling by batch rectification—Possibilities to really reduce a needed evaporation

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Today energy costs are very high with a tendency to rise. As about 2260 kJ are needed to evaporate one liter of wort, it is advantageous to reduce the total-evaporation during the boiling of wort. The needed total-evaporation to undershoot a required target-concentration in the cast out wort is a property of the solution and is given by the vapor-liquid-equilibrium of unwanted flavors in wort and the start-concentration in the kettle-up wort. Thus, this total-evaporation cannot be reduced by existing wort boiling systems, although this is often asserted. The only possibility to reduce the needed overall evaporation is a fractional distillation/rectification. Based on the basics of evaporation and rectification, a new wort boiling system was constructed. In this new rectification wort boiling system, a side stream of wort is constantly drawn from the wort kettle and fed into a rectification column. This column is connected to the kettle. Thus, the evaporating vapor and the recirculating wort are in strong contact. In this way, unwanted flavors are strongly enriched in the evaporating vapor. Because of this, the discharged steam has a concentration of unwanted flavors that is much higher than the one produced by normal boiling systems. Test trials were performed with the new wort boiling system and its evaporation efficiency (AE) was acquired. The results of the measurements show that an equal reduction of unwanted flavors is achieved with about 50% less overall evaporation then the one a normal wort boiling system needs. The evaporation efficiency (AE) is thus doubled. With this new wort boiling system it is now possible to really reduce the needed overall evaporation at the batch process stage of wort boiling. More then 50% (!) of the overall evaporation can be reduced in comparison to the existing wort boiling systems although the aroma profile of the resulting wort stays equal. If the boiling time is also reduced, the resulting worts have a clearly lower thermal stress and a better protein composition. The needed column can be exchanged in nearly every existing wort boiling system. The savings of energy and money are enormous.

P-89

Process engineering fundamentals to remove ambiguity within the scope of wort boiling

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In modern times many assumptions about the boiling process and especially about evaporation that are circulating in the brewing community are erroneous. This partial lack of knowledge can be found throughout the various groups of people involved in the brewing process; brewmasters, as well as manufacturers of brewing plant equipment. One of the main misconceptions is that the efficiency of the evaporation of undesired flavors, such as dimethyl sulfide (DMS), can be increased by creating a larger surface area of the boiled wort. A larger surface area can only increase the velocity of the evaporation but it cannot, on any account, decrease the overall evaporation. The latter is given by the concentration of a flavor in the wort before boiling, by the target concentration after boiling and by the vapor liquid equilibrium (VLE) of the aroma compound in wort. Another important misconception is that the wort matrix is so complex that the VLE of flavors could differ in every wort and thus cannot be generally described. In truth, the VLE of a flavor in wort can be described as VLE of the same component in pure water. This is due to the fact that flavors are present in wort in such small amounts that each flavor molecule is only surrounded by water molecules (infinite dilution). Thus flavors cannot interact among each other. This is also valid for possible interactions of flavors with the other solutes, especially sugars. Finally brewers do not distinguish between the different types of evaporation, although the calcination of flavors underlies different mechanisms at an atmospheric boiling of wort or at a flash evaporation. The fact is that the highest enrichment of a flavor in steam vapor, and thus the minimum required evaporation, is reached by a normal atmospheric boiling procedure. The different types of flash evaporations can only reduce this enrichment in the steam and thus increase the necessary total evaporation time in comparison to an atmospheric boiling of the same wort but not vice versa. All these misconceptions show, that there is a huge lack of knowledge of the process engineering essentials of the wort boiling process in the brewing community. Therefore the process engineering basics of a boiling process will be explained in detail. This includes answers to the following questions: Why does a liquid (wort) boil? What is a vapor liquid equilibrium? What is the difference between atmospheric boiling and flash evaporation? What are we talking about when we discuss various residue curves? This basic knowledge enlarges the technological understanding in the brewing community and helps to critically review perceptions concerning wort boiling that have been taken for granted.

P-90

Differences in the evaporation efficiency (AE) of common wort boiling methods and their effects on the resulting wort

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An important function of the boiling of wort is the calcination of flavors. If the total-evaporation is insufficient off-flavors will occur in beer. Thus a brewer is anxious to have an adequate overall evaporation. Brewers have recognized, that the efficiency of the calcination of flavors differs in varying wort boiling systems. As no one has an explanation for these differences, people in the brewing section claimed that wort is too complex and that the fluid mechanics in a boiling system cannot be clarified, so that this efficiency could differ in every wort boiling system in any brewhouse for each brew. (It is shown elsewhere that this is not true!) That is the reason why total-evaporation is oversized in most breweries. Because of this a dimensionless index was established, the so called evaporation efficiency (AE). With this index every wort boiling system can be characterized and classified. One disadvantage of this method is that the AE has to be measured experimentally. Because of this the different evaporation mechanisms that underlie the different boiling systems were researched in this work. Based on the basics of evaporation, residue-curves were established for every common wort boiling system under different conditions (temperature, pressure). These residue curves have been confirmed with experimental decreasing values of flavors during wort boiling in various boiling systems. It was possible to show that some boiling systems perform a mixture of different types of evaporation. Thus the decrease of a flavor component does not succeed the classical type of residue curves. That could be the main reason why it was misleadingly claimed that the calcination of compounds in wort is too complex to be predicted. It is shown that the decrease of different wort flavor components can be preeminently predicted with the calculated residue curves for every common boiling system. With these formulas it is now possible to predict the AE for every wort boiling system under every condition. Thus an experimental determination of the AE can now be avoided. It is now also possible to predict a needed overall evaporation individually for every brew in different boiling systems. An important result is, that the AE can differ strongly with the different common boiling technologies. Because of this the common boiling systems are suitable for the calcinations of flavors in a different way. As a result all common wort boiling technologies are classified based on their efficiency. If the efficiency of a boiling system is known, it is much easier for brewers to decide which boiling system is the most qualified for their applications. Furthermore the efficiency of a wort boiling system can differ under different conditions. Because of this, existing wort boiling systems can be highly optimized if some parameters are adjusted correctly. With this new knowledge this is now possible!

P-91

A new method to reduce the recreation of off-flavors during the whirlpool rest

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A new approach is presented to reduce the content of undesired flavors during the whirlpool rest by increasing the vaporization rate. In this case it is not the thermodynamic effect of evaporation that is essential, but in fact the process of vaporescence. This is realized by constantly evacuating the air above the wort in the whirlpool during the whirlpool rest. With shortened boiling processes, the conversion of precursors of undesired flavors is often insufficient. Thus, off-flavors are continuously produced even during the whirlpool rest and cannot be reduced without an additional evaporation, even with modern practices. The most important flavor in this context is dimethyl sulfide (DMS). With the presented method an increase in the vaporization rate is created by constantly evacuating the air above the wort in the whirlpool. Test trials have been carried out in a pilot-plant unit which was equipped with suitable mountings to permanently exhaust the air above the wort during the whirlpool rest. Thus, a comparison by gas chromatography between the normal rested wort and the treated wort was conducted. Samples were taken in triplicate at different times during the whirlpool rest, to meet statistical requirements. For this, the wort was analyzed before and after a whirlpool rest of 30 min., with and without the new suction system. To show that the differences in the resulting amounts of DMS are mainly caused by the increase of the vaporescence, trials were also performed with DMS in pure water, where no production of DMS can occur. The results demonstrate that there is a significant effect of using suction equipment on the content of DMS after the whirlpool rest. This is solely due to the higher vaporescence. Because of the high saturated vapor pressure of DMS in combination with its activity coefficient, the mass stream of DMS can be significantly enhanced by permanently exhausting the air above the wort during the whirlpool rest. This results in a lower concentration of DMS in the wort after the whirlpool rest. The influence on other components (e.g. the desirable hop component linalool) is not significant, because of their lower vapor pressures and activity coefficients. Shorter boiling times and a higher amount of precursors did not influence the quality of the resulting wort while using the investigated suction system. A patent has been assigned based on these results.

P-92

Prediction of malt sugar content in converted mash taking into account the particle size distribution of starch

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The central process of beer production is the mashing process. In this step ground malt and water are mixed and a suspension is produced. By using special time and temperature profiles this mash is transformed to a solu-

tion which is called wort. This wort is the essential basis for the quality of the final beer. For every brewer this process is of highest interest. Different enzymatic reactions have to be researched. The most important one of them is starch degradation and along with it the creation of sugars. Working amylolytic enzymes and their conditions are well known and can be found in the literature. In spite of this the kinetics of the whole process, which consists of gelatinization and saccharification, is difficult to describe. One reason can be seen in the influence of the particle size distribution of starch. The missing knowledge results in missing control which can be seen in standard, safety-mashing programs. Predicting the kinetics of that step would be one possibility for improving the whole process by optimizing time, energy and use of raw materials. The aim of this work is to show the possibility of the prediction of the content of sugars and dextrins at the end of the mashing process using a calculation method. Therefore a new version of a calculation model by Einsiedler is presented, which is matched to pure component systems and adapted to mashes. It will be seen that this new method is an improved tool for predicting and optimizing mashing results.

P-93

The use of response surface methodology to optimize malting conditions of quinoa (*Chenopodium quinoa* L.) as a raw material for gluten-free foods

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Celiac disease is a condition in which the person's body reacts to the gliadin fraction of wheat and the prolamins of rye (secalins), barley (hordeins) and possibly oats (avidins) The incidence of celiac disease, or other allergic reactions/intolerances to gluten, is increasing largely due to improved diagnostic procedures and changes in eating habits. Currently it is estimated that 1 in 100 of the world's population is suffering from this condition. This implicates a high demand for high quality gluten-free products. In this study quinoa (*Chenopodium quinoa* L.), which belongs to the species goosefoot, which is regarded as gluten-free, was used as the raw material. The objective of this study was to optimize the malting conditions to produce a gluten-free malt of high quality for gluten-free foods and beverages. Response surface methodology was used to investigate the influence of the three malting parameters, vegetation time, degree of steeping and temperature, on the quality of quinoa malt. Each predictor variable was tested at three levels. Vegetation times were 5, 6 and 7 days, degrees of steeping were 46, 50 and 54% and vegetation temperatures were 8, 11.5 and 15°C. Kilning temperatures of 65°C were used. The used analyses were based on methods outlined in EBC or by MEBAK. The raw material was yielded in 2005 in Bolivia. A range of malt quality parameters was determined including extract, apparent attenuation limit, gelatinization temperature, α-amylase activity, β-amylase activity, limit dextrinase activity, Kolbach index, α-amino nitrogen, viscosity, and color. The optimal malting program was achieved with 5 d of vegetation time, 46% degree of steeping and 15°C steeping and germination temperature. The obtained amylolytic and proteolytic attributes were 59.6% [d.m.] extract, 90 U/g β-amylase activity, 2022 U/kg limit dextrinase activity, 930 mg/L soluble N, and 19.2 mg/100 mL FAN. This publication shows clearly that on the one hand RSM is a proven method for testing the malting conditions of unknown cereals, and on the other hand *Chenopodium quinoa* is a crop with potential as a raw material for malting purposes. Furthermore, it is feasible to create foods like breads, fermented beverages and beer from cereals without affecting the quality of life of patients with celiac disease.

P-94

The use of response surface methodology to optimize malting conditions of oat (*Avena sativa* L.) as a raw material for alternate fermented beverages

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Oats (*Avena sativa*) are one of the most popular cereals for human consumption. In the middle ages oats were the brewing cereal par excellence. Over the centuries they were substituted for with other cereals, and their brewing properties were nearly forgotten. Today oats are popular once more because of their excellent health-related properties. For people who suffer from celiac disease oats are also of interest. Based on their historical use in brewing and their health-related properties pilot malting trials were carried out with different cultivars, followed by brewing trials with a selected cultivar. The results obtained showed that oat malt is an appropriate choice for brewing. Response surface methodology was used to investigate the influence of three malting parameters, vegetation time, degree of steeping and temperature, on the quality of oat malt. Each predictor variable was tested at three levels. Vegetation times were 6, 7 and 8 days, degrees of steeping were 42, 45 and 48% and vegetation temperatures were 12, 15 and 18°C. A kilning temperature of 80°C was used. The analyses used were based on methods outlined in EBC or by MEBAK. The raw material was yielded in 2007 in Granskevitz, Germany. A range of malt quality parameters was determined, including extract, apparent attenuation limit, gelatinization temperature, α-amylase activity, β-amylase activity, limit dextrinase activity, Kolbach index, α-amino nitrogen, viscosity, and color. This publication shows clearly that on the one hand RSM is a proven method for testing the malting conditions of unknown cereals, and on the other hand *Avena sativa* is a crop with potential as a raw material for malting purposes.

P-95

Optimization of germination time and temperature for malting of oat using response surface methodology

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The use of oat as a brewing raw material has gained more and more interest recently in the brewing industry as it leads to beers with a particular taste and flavor, as well as being considered a niche product for celiac sufferers. Oat is known for its health-promoting properties due to its high contents of soluble dietary fiber, a large selection of vitamins, minerals, sterols and antioxidants. It has been reported that bioprocessing such as malting can increase the amount of bioactive compounds present in grains, only a small proportion of oat has previously been malted. To our knowledge there is no study available that deals with the optimization of the germination process with regard to beer production. The objective of this work was to study the impact of germination conditions on the brewing performance of oat malt. Response surface methodology (RSM) was used as a mathematical tool to understand the interactions between the process and quality parameters. Oat was malted in a micro malting machine. While steeping and kilning conditions were held constant, the duration of the germination stage and the germination temperature were varied. The malts were analyzed for the activities of amylases and β-glucanase and the content of nitrogen. The malts were mashed following the Congress mashing regime, and the mashes were analyzed for extract content, fermentabililty, content of soluble nitrogen (SN) and free amino nitrogen (FAN) as well as viscosity. While the variations in the germination temperature did not significantly affect any of the analyzed malt properties, the length of germination period had a crucial impact on many parameters important for brewing. Most pronounced were the changes in the activities of α- and β-amylase and the fermentability of the mash. The RSM models calculated maximal fermentability, which was even higher than usually found in barley malt for a germination time of 116 h, which coincides with the point of maximum α-amylase activity. The overall enzyme activity was lower than normally found in malted barley. SN and FAN increased with prolonged germination time, while total nitrogen, extract content and viscosity were not significantly influenced. A problem in the use of oat malt for brewing might be the low content of extract, which was approximately 75% of that expected for barley malt. However, the oat malt with optimized malting conditions had potential as a raw material for brewing since the majority of the relevant malt properties resemble the ones of barley malt. Oat malt might also be used as an ingredient for functional beverages or for applications in the baking industry.

P-96

The use of response surface methodology to optimize malting conditions of triticale (×*Triticosecale* Wittmack) as a raw material for alternate fermented beverages

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Triticale (×*Triticosecale* Wittmack) is not artificial. It is a hybrid of wheat (*Triticum*) and rye (*Secale*) first bred in laboratories during the late 19th century. As a rule, triticale combines the high yield potential and good grain quality of wheat with the disease and environmental tolerance (including soil conditions) of rye. Only recently has it been developed into a commercially viable crop. The word 'triticale' is a fusion of the Latin words *triticum* (wheat) and *secale* (rye). The primary producers of triticale are Germany, France, Poland, Australia, China and Belarus. Triticale is mainly used as an animal feed grain. Response surface methodology was used to investigate the influence of three malting parameters, vegetation time, degree of steeping and temperature, on the quality of triticale malt. Each predictor variable was tested at three levels. Vegetation times were 5, 6 and 7 days, degrees of steeping were 42, 45 and 48% and vegetation temperatures were 15, 18 and 21°C. A kilning temperature of 80°C was used. The analyses used were based on methods outlined in EBC or by MEBAK. The raw material was yielded in 2007 in Granskevitz, Germany. A range of malt quality parameters was determined including extract, apparent attenuation limit, gelatinization temperature, α-amylase activity, β-amylase activity, limit dextrinase activity, Kolbach index, α-amino nitrogen, viscosity, and color. This publication shows clearly that on the one hand RSM is a proven method for testing the malting conditions of unknown cereals, and on the other hand triticale is a crop with potential as a raw material for malting purposes.

P-97

Sensory and analytical characterization of top fermented beer brewed out of 100% buckwheat malt

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Buckwheat (*Fagopyrum esculentum*) is an annual melliferous crop of the Polygonaceae family. It is a rich source of starch, protein and dietary fiber. Small scale mashes with 100% malted buckwheat revealed its potential as a gluten-free brewing material. Lab scale optimization of buckwheat's malting regime and mashing program resulted in more favorable brewing attributes: higher values of extract recovery, higher levels of amylolytic enzymes, higher values of free amino nitrogen (FAN) and total soluble nitrogen (TSN). Further work on the addition of industrial enzyme preparations to 100% buckwheat mashes revealed higher values of total fermentable extract (TFE), fermentability, FAN, TSN and decreased wort viscosity. In this study a sensory and analytical characterization of a top fermented beer brewed from 100% buckwheat malt is presented. Malting was carried out on a 200 kg pilot scale using lab scale optimized procedures for steeping, germination and kilning, while the brewing trials were carried out in a 1000 L pilot research brewery. Difficulties with mashing and filtration were encountered during the brewing process, which resulted in problems during fermentation and beer filtration. However the resultant beer was characterized according to standard beer methods. Levels of aliphatic alcohols, volatile esters, maturing indicator compounds and aroma compounds in the final beer were determined using gas chromatography (GC). Taste testing was carried out according to the Deutsche Landwirtschaftsgesellschaft scheme. The beer was evaluated in fresh and forced aged states for the following attributes: odor, purity of taste, mouthfeel, tingling and bitterness. In addition, the beer was examined based on maturing compounds. Analytical results indicate that buckwheat beer compares quite closely to a typical wheat beer with regard to color, pH, TSN, FAN, fermentability and total alcohol. However, the extract of the buckwheat beer was lower, resulting in a final extract yield of 44.5%. GC analysis of the resultant beer reveals commonly encountered levels of esters. A low level of fusel alcohols in comparison to a typical wheat beer was detected along with a low level of γ-nonalactone. However, a high level of ethyl caprinate (coconut flavor), along with a high level of lauric acid (fatty odor) was detected. Sensory analysis indicates that the buckwheat beer was acceptable with regard to odor, purity of taste, mouthfeel, tingling and bitterness. In conclusion, results of this study prove buckwheat's qualification as a gluten-free brewing material, and with process optimization, its readiness for marketing.

P-98

Brewing with 100% unmalted sorghum—Introduction of a novel adjunct mash regime using heat stable bacterial endopeptidase

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It is essential to use exogenous enzymes for 100% raw sorghum brewing. Conventionally, the mashing regime includes separate proteolytic, liquefaction and saccharification steps at different temperatures with enzymes that might include endopeptidase, heat stable α-amylase, fungal α-amylase and/or glucoamylase and glucanase/xylanase preparations. The endopeptidase is typically the neutral metalloendopeptidase (bacillolysin) from the mesophilic *Bacillus subtilis*. This enzyme catalyses the hydrolysis of protein into soluble nitrogen and peptides but is limited to operational temperatures of up to 60°C. By using a heat stable neutral metalloendopeptidase (thermolysin) from a strain of *Geobacillus caldoproteolyticus* we can eliminate this proteolytic rest in the mash, and we have characterized the effect in terms of nitrogen solubilization, extract, fermentability and mash filtration. All mashes, using unmalted sorghum, were carried out, using a standardized cooking and mashing protocol, at a laboratory scale at Danisco, Stockport, and at a pilot scale at Ziemann Ludwigsburg GmbH (10 hL scale). Standardized methods were used to determine sorghum composition, extract, fermentability, spent grain starch, and to measure wort separation performance. Wort and beer carbohydrate, free amino nitrogen and alcohol concentrations were measured by HPLC. The mash application of the heat stable neutral metalloendopeptidase with a shortened mash regime demonstrated, with a 80°C liquefaction step, equivalent performance in increasing FAN levels compared with the conventional neutral metalloendopeptidase process. However, the process for the latter required a proteolytic rest of 60 minutes at 60°C prior to the liquefaction step. There was no negative effect on mash filtration when using the heat stable neutral metalloendopeptidase, and depending on overall enzyme composition and dose rates, an increase of fermentability of up to 82% real degree of fermentation (RDF) was observed. Heat stable neutral metalloendopeptidase may be used in a 100% unmalted sorghum mash to increase free amino nitrogen and peptide levels, ensuring good fermentation. Moreover we have demonstrated that the use of this enzyme may significantly reduce mashing time without compromising performance in terms of extract, RDF, wort or beer quality.

P-99

Antioxidant activities and phenolic profiles of millet wine during storage

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Cereal wine has been claimed to have health-promoting effects, which may be related to antioxidant activity. This research presents information related to the flavonoid and total phenol contents of millet wine stored for different periods (1, 4, 6, 8, or 10 months) at 4°C and their antioxidant activities, including linoleic acid peroxidation inhibition, reducing powder, ferrous ion chelating capacity, and scavenging activity on the free radical DPPH during storage compared with synthetic antioxidants, such as α-tocopherol and BHA. Moreover, twelve phenolic compounds, including gallic acid, protocatechuic acid, gentisic acid, (+)-catechin, vanillic acid, caffeic acid, syingic acid, (–)-epicatechin, ρ-coumaric acid, ferulic acid, cinnamic acid, and quercetin in millet wine were identified by HPLC. This study aimed to compare the antioxidant activities of millet wines stored during different periods and to analyze phenolic constituents for their contributions to antioxidant activities. Results indicated that millet wines ex-

hibited noticeable antioxidant activities and that linoleic acid peroxidation inhibition reducing powder were highly correlated with total phenol contents. Moreover, DPPH free radical scavenging activity and reducing powder were highly correlated with flavonoid contents.

P-100

The use of response surface methodology to optimize malting conditions of teff (*Eragrostis tef* L.) as a raw material for gluten-free foods

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Celiac disease (CD) is a condition in which the person's body reacts to the prolamins of wheat, rye, barley, and oats. The only way to treat CD is a total lifelong avoidance of gluten consumption. In this study teff (*Eragrostis tef* L.), which belongs to the family Poaceae, which is regarded as gluten-free, was used as raw material. The objective of this study was to optimize malting conditions to produce a gluten-free malt of high quality for gluten-free foods. Teff, with a thousand kernel weight of 0.3–0.4 g, needed special arrangements like small sieves etc. Teff has a remarkable agronomic advantage because its water requirement is probably the lowest of any major cereal. Response surface methodology was used to investigate the influence of three malting parameters, vegetation time, degree of steeping and temperature, on the quality of teff malt. Each predictor variable was tested at three levels. Vegetation times were 1, 2 and 3 days, degrees of steeping were 44, 50 and 52% and vegetation temperatures were 18, 21 and 25°C. A kilning temperature of 65°C was used. The analyses used were based on methods outlined in EBC or by MEBAK. The raw material was yielded in 2006 in Utah, USA. A range of malt quality parameters was determined including extract, apparent attenuation limit, gelatinization temperature, α-amylase activity, β-amylase activity, limit dextrinase activity, Kolbach index, α-amino nitrogen, viscosity, and color. This publication shows clearly that on the one hand RSM is a proven method for testing the malting conditions of unknown cereals, and on the other hand *Eragrostis tef* is a crop with potential as a raw material for malting purposes.

P-101

The use of response surface methodology to optimize malting conditions of two black rice varieties (*Oryza sativa*) as a raw material for gluten-free foods

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Response surface methodology was used to investigate the influence of three malting parameters, vegetation time, degree of steeping and temperature, on the quality of rice malt. Each parameter was tested at three levels. Vegetation times were 6, 7 and 8 days, degrees of steeping were 38, 41 and 44% and vegetation temperatures were 20, 25 and 30°C. All analysis methods were based on methods outlined in MEBAK. A range of malt quality parameters was determined, including extract, apparent attenuation limit, gelatinization temperature, α-amylase activity, β-amylase activity, limit dextrinase activity, Kolbach index, α-amino nitrogen, soluble nitrogen, viscosity, and color. The optimal malting program was achieved with 8 d of vegetation time, 44% degree of steeping and 30°C steeping and germination temperature. Under the optimal conditions the corresponding predicted malt qualities of black normal rice and black sticky rice malt were 61.5 and 58.5% [d.m.] extract, 117 and 100 mg FAN/100 g malt, 375 and 290 mg soluble N/100 g malt, 72 and 74 U/g α-amylase activity, 76 U/g and 59 U/g β-amylase activity, 3,861 and 3,972 U/kg limit dextrinase activity, respectively.

P-102

Comparing different rinsing methods during cleaning in place of process vessels to minimize water use

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Process vessels such as fermentation or aging tanks are usually equipped with stationary spray balls or rotating spray heads to dispense both the detergent solution and water rinses during the cleaning in place (CIP) procedure. Rinsing removes soil or traces of detergent and is carried out either by spraying water continuously or by a series of short water bursts until the tank is free of any contamination. Rinsing during CIP can consume large quantities of water. Water conservation and the cost of water are important incentives for breweries to find ways to minimize its use. Theoretical modeling of continuous versus burst rinsing through stationary spray balls shows that burst rinsing can be considerably more efficient in the use of water. The operation of typical rotary head machines is also analyzed qualitatively to determine their effectiveness during rinsing, especially compared with the stationary spray balls. Such analysis can help to make predictions for minimizing water use.

P-103

Design for success—Proper brewhouse steam jacket selection

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One of the harshest environments for equipment in the brewery is in the brewhouse. Cyclical steam cycles, caustic applications and operational stresses of a batch process present challenges for vessel designers. This paper will review current design criteria, heat transfer alternatives, root cause analysis of failures and cost comparisons of brewhouse heating surfaces. The heat transfer design criteria of brewhouse vessels is well known. Brewkettles, mash tuns, cereal cookers, and hot water tanks all utilize some type of heat transfer equipment to heat their contents to the brewer's desired recipe. There are many different types of equipment available to achieve this heat transfer. A review of heat transfer equipment types used in various process industries was compared to what is currently used in brewhouse applications. Various steam jacket designs and construction techniques were studied. Actual cases of premature failure were investigated and a failure analysis prepared. Pareto principles were applied to address the root cause of these deficiencies. The results led to a better selection of modern alloys, proper insulation specifications, and structural and mechanical designs that extend vessel service life. Significant brewhouse production costs, as well as repair or replacement costs, can be avoided by incorporating the best practices described in this presentation.

P-104

Understanding the value generated from achieving tighter temperature control of process water through the use of new technology

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Reducing process variability was highlighted in a 2001 ARC study as a focus for maximizing in the future. Process variability surrounding the use of water, the most precious resource, has gained attention in the triple bottom line economy as the true cost of water is understood. The paradox that businesses face is that many manufacturers frequently install the latest technology for control systems, yet insist on antiquated valve technology in processes because "if it isn't broken, why fix it"? Little has changed in the process of blending water, with an acceptance of 30 year old valve design, pneumatically actuated, externally controlled using independent monitoring and control logic. Incremental improvements in components have delivered little change in process performance. Recent developments in shear action swirl mix valves, combined with high resolution electronic actuation and fast response temperature probes, enable true high speed closed loop control. This fundamental step change in performance can deliver sustainable value to organizations in water, energy and maintenance savings. These new valves have shortened the mixing zone from what was accepted as 8 to 15 pipe diameters from the outlet of the valve to the point

of monitoring to complete mix at the valve. This reduction provides value in both reduced volumetric quantity variance, as well as improved time function. Traditional pneumatic actuation relies on assumed constant air pressure and quality, maintained through energy intensive compressors, driers and maintenance intervention. Electro pneumatic positioning has improved, but is still reliant on assumed air pressures and quality. Technology is now available in the form of high speed and resolution electronic stepping motors combined with high torque planetary gear trains that provide speed equal to pneumatics, but resolution and accuracy that is unmatched. High speed, negative temperature co-efficient NTC probes that deliver greater accuracy than resistance temperature detector (RTD) probes are now integrated as part of these actuator and valve packages, including configurable closed loop temperature control software, and deliver a new a standard of accuracy. The cost of process variance to companies is apparent in a number of forms, both tangible and tacit. The direct cost of water and the gas bill are tangible measures. The cost of wasted energy can be calculated using the specific heat equation and can be observed in many control rooms as the area under the actual line on temperature monitoring graphs that is above the temperature set point. Innovative use of technology to reduce the use of resources will drive robust economic growth and meet sustainability demands into the future.

P-105

Sustainability practices in brewing and packaging—Impact of sanitation programs on overall water consumption

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Many brewers monitor the ratio of water consumption to beer production as a measure of process efficiency. CIP cleaning and sanitizing accounts for a significant portion of the water used in brewing operations. This paper will review water consumption at several large breweries. It will compare sanitation-related water consumption in brewing and packaging. Recommendations are made to optimize water consumption in cleaning and sanitizing, through innovative cleaning chemistry, CIP programming and engineering.

P-106

Availability and quality of water: Addressing future problems with modern water treatment technologies

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It is generally known that the access to fresh water in sufficient quality and quantity is poor in some parts of the world. Lack of decent well water forces humanity to look for other sources, like surface water. As surface water quality in general is much more influenced by humans and subject to seasonal changes, new challenges have to be met by water treatment technology. This tendency has gripped the food and beverage industries as well, where sometimes the supply of water has already become the crucial location factor. This presentation describes a new approach for dealing with surface water in brewing by means of an example of a brewery located on the banks of Lake Victoria in Africa. The brewery sources its water directly out of Lake Victoria and was formerly treated with sand filters, removing parts of turbidity and organics. Due to intense human usage, water levels have dropped in recent years. In parallel, water quality deteriorated, especially during rainfall, making it impossible to provide decent water quality from the existent water treatment plant. The approach was to use ultrafiltration instead of sand filters. The advantages of ultrafiltration are the complete removal of particles/turbidity and bacteria as well as viruses. Therefore ultrafiltration forms an effective germ barrier, which is essential for the food industry and may be reached naturally only by filtration through different soil layers over a decent period of time. Before commissioning, intensive pilot trials over a period of six months were carried out in order to determine the optimized operation and cleaning procedures as well as the yield of the plant. Results of the piloting will be presented. Surface waters generally possess low hardness, which makes it necessary to add non carbonate hardness in order to adapt the ionic composition of the water for brewing purposes and a client's specifications as well. Therefore further treatment consists of a CALMIX® plant, which forms $CaCl_2$ and $CaSO_4$ in totally dissolved form out of lime, hydrochloric acid and sulfuric acid. The combination of ultrafiltration with CALMIX® proved to be a very cost effective solution for the conditioning of heavily used surface water to brew water. Operation data from the large-scale water treatment plant will be presented in detail. It shows how future problems like the usage of surface waters for food production may be addressed using modern water treatment techniques. The adoption of new water treatment techniques will play a key role in technologies.

P-107

Biofouling and process cleaning: A practical approach to understanding what's happening on the walls of your pipes

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Biofouling is a ubiquitous problem to a great many industrial processes, including brewing and other industrial fermentation processes. Biofouling in process equipment and water systems can lead to product quality incidents as well as process problems. In spite of their widespread presence in industrial systems, biofilms can be difficult to detect and even more difficult to control. Biofouling control in industrial systems is linked strongly to the biofilm's exopolymer, which in turn is influenced by product chemistry, system design, system hydraulics and cleaning process parameters. This paper describes a practical approach to detecting, characterizing and controlling biofouling in brewing and other full-scale fermentation processes.

P-108

The relationship between water consumption and energy usage in the malting and brewing industries: Opportunities and priorities

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This poster will discuss improving environmental performance in malting and brewing facilities from the point of view of interconnections between water efficiency and energy efficiency. Both malting and brewing processes are energy-intensive and large consumers of water. As well as charges for carbon dioxide release and fuel and water prices increasing, there is legislative and public pressure to act in a more sustainable fashion. The chemical engineer should consider the environmental impact of the plant in a holistic fashion to make sustainable modifications or investments. The largest users of energy in the two industries are malt kilning, wort boiling and packaging (excluding transportation). The largest water usages are steeping in the malting process and packaging and cleaning in brewing (excluding the water in the product). End-of-pipe technologies that recycle much of the water that would otherwise be sent to effluent treatment may have a high energy demand. For example, recycling of a maltings' steep water is potentially a very large water saving, but any reprocessing will have a cost in energy. However, these technologies must be appraised against dwindling water resources, the costs and environmental impact of the alternative effluent treatment and discharge and the likely future price of potable water. Water must be considered as much more valuable when it is hot water; therefore, recovery of heat, water and chemicals from in-place cleaning processes must be increasingly prioritized. Similarly, other cleaning strategies include increased use of lower temperature cleans and monitoring through automation to minimize cleaning intensity. The costs of energy and water purchase will be higher in the future; therefore, projected increases should be factored in to technology decisions that are made today. It is possible to assess the sensitivity of capital expenditure to different levels of price rise. Clearly local factors such as the availability of water will affect any particular decision. The packaging line is a major user of energy and water, and there are technological opportunities for improvement. The choice of package itself will affect the environmental impact; however, clearly package formats are enormously influenced by retailer and consumer demands. This poster helps to highlight priorities for both the site engineer and strategic planner in developing the process in a suitable manner for the current commercial environment.

P-109

Valuable water

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Water, and more precisely clean water, is one of the most precious goods we have on earth. Without water, there is no life and certainly no beer. Clean water is already a scarce good in large parts of the world. Pollution of the ground and surface water prohibits the use of the water without extensive cleaning. In the European Union we see different regions where the consumption of clean water has to be restricted because of shortages, especially in summer. In the near future there will be more countries with the same problem as, for instance, Spain and Belgium, which results in the restriction of fresh water consumption more often. This will have an impact on households but also on industries that depend on clean fresh water. Those industries will have to think about water management. As discussed at the last WBC in San Diego, water reduction in the brewery is the first step in reducing the fresh water consumption of a brewery. Re-use of relatively clean wash water from the last CIP cycle, more effective cleaning cycles and so on, should be considered. But, in the end there is a point where these measures have been implemented, and there is no room for more direct recycling left. Then, the re-use of the used water needs a little help. Cleaning of this process/wastewater is the way to go. In a lot of breweries a wastewater treatment plant, or better a process water treatment plant, is already installed. Not only to meet the discharge requirements, but also for the production of valuable methane. To re-use water a membrane filtration unit has to be installed. With the right set up, demineralized water can be produced. This water is very suitable for boiler feed water, as make-up water for chemical dilution or as wash water. For a brewery with a water consumption of 3 hl/hl beer, a further reduction of fresh water consumption with 0.7 hl water is feasible. This paper will describe the way to reduce fresh water consumption.

P-110

Application of substrate specific enzymes and bottle-washing-lye for dwell time reduction during anaerobic digestion of spent grains

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Against the background of rising energy costs and limited options for waste disposal, organic brewery residues (spent grains, malt dust, hot/cold break, surplus yeast/sediments, labels, kieselguhr sludge, wastewater) offer an interesting way of energy recovery. Due to the inhomogeneous chemical waste composition and the various solid, pasty and liquid compounds, anaerobic digestion (AD) represents the most advantageous technology and is, in the case of wastewater treatment, already state-of-the-art. Concerning the chemical composition of spent grains there are still problems in hydrolyzing the lingo-cellulose fraction, which consists of hemicellulose, cellulose and lignin, in a short time. The performance of hydrolytic bacteria without any pre-treatment is not enough to achieve economically advantageous dwell times. Therefore the effectiveness of a substrate specific enzyme mixture as well as alkaline treatment were tested in 20 l reactors in laboratory scale. The experiments were operated in a two stage fermentation system (first stage: hydrolysis in continuous stirred reactors; second stage: methanation in fixed bed reactors) with liquid-solid-separation in between. To evaluate the degradation of spent grains the volatile fatty acid (VFA) concentration, chemical oxygen demand (COD), pH-value and ammonium concentration of the liquefied fraction, as well as the content of fat, protein and fiber of the residual solid fraction, were analyzed in duplicate. The composition of the produced biogas was analyzed by gas chromatography and mass spectrometry (GC/MS). For inducing hydrolysis, seeding sludge of a municipal wastewater treatment plant was used as inoculum. By the application of substrate specific enzymes it was possible to force the liquidation-time of the complete protein and fat fraction of spent grains. The additional added enzymes also quickened the microbial hydrolysis of the lingo-cellulose fraction. However, the dwell time reduction by substrate specific enzymes was not extensive enough, for which reason alkaline treatment of the remaining lingo-cellulose fraction was tested by adding bottle-washing-lye. Within a few hours the hemi-cellulose fraction was completely liquefied. The remaining cellulose was treated enzymatically again. After each hydrolytic step liquid-solid-separation took place. The liquid fraction was subsequently fermented in a fixed bed reactor. Thereby the degradation of hydrolytic products to biogas was analyzed. During the experimental stage no hindrance of the methanation could be detected. By the application of substrate specific enzymes in combination with alkaline treatment with bottle-washing-lye the dwell time of spent grains during anaerobic fermentation could be reduced below 12 days. An economic process could be proven.

P-111

Responsible tank cleaning—The blueprint for the future

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Massive savings in water, energy, detergent and wastewaters are achievable when breweries approach brewhouse, fermentor, aging and yeast tank cleaning as a partnership with the equipment supplier and detergent specialist, from the design to operational phases. It is now essential rather than desirable for breweries to demonstrate true environmental commitment in their cleaning systems. The latest developments in detergent formulations matched to specific cleaning head designs are one example of the opportunities available. Practical comparisons of before/after studies will be given. Concern for guaranteed hygiene in cleaned tanks will be addressed and the decreasing need for manual checking of cleanliness will be reviewed. This paper will provide best practice guidance on a systematic approach to *responsible* tank cleaning.

P-112

Control of utilities water treatment systems using automated chemical feed verification

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Reliable accurate control of treatment chemicals for boiler, cooling and other utility water systems is essential to efficient and dependable plant operations. While plant operations become more and more automated to ensure production reliability, chemical feed systems in general still tend to be based, at best, on an operator wet chemistry test performed at some routine interval. Then, an adjustment to a metering pump or chemical feeder is made to compensate for test levels. In reality a sudden loss of treatment chemical feed to a boiler, cooling water or evaporative condenser system will not shut down plant production. However, routine unchecked interruptions in chemical feed over extended periods of time can cause internal corrosion and/or deposition, which like a cancer will fester and grow over time, possibly culminating in a catastrophic failure halting production and potentially resulting in a plant safety event. This study compares the differences of a traditional manual controlled chemical feed system to that of various levels of automated chemical feed systems on cooling water loop, its metallurgy corrosion rates and throughput efficiencies. A cooling water system at a large brewery consisting of an industrial size cooling tower and several evaporative condensers was monitored over a period of time. First, treatment chemicals were fed into the makeup water of this system by a manually adjusted chemical metering pump. Adjustments to this pump were made based on operator wet chemistry tests results. Next the metering pump was connected to a makeup water flow meter so that chemical feed rates would be proportionally ramped up and down based on system flow. Finally an automated chemical dosing system was installed which would accept a signal from the makeup water flow meter and then accurately measure and adjust chemical dosing rates to maintain a constant PPM dosing rate. Routine monitoring of actives based chemical levels and overall system corrosion rates and heat transfer rates were recorded and evaluated. At the same time feedback from this controller was brought into the central utilities plant control room giving operators another diagnostic tool and control over their critical process systems. The end result shows that while acceptable overall system control can be maintained with a manual feed system, the relatively small cost associated with automation of water treatment chemical feed systems can exponentially pay for themselves with cleaner more efficient systems. This will result in longer run times between system cleanings and turnarounds while improving system efficiency, reducing power consumption and improving plant safety. In short this is one of the least expensive insurance policies in which your plant can invest.

P-113

Visual recording of process control interfaces

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The automated process control of industrial or laboratory brewing plants is normally performed by PLC and man/machine interfaces on a personal computer. The visualization includes process graphics and records the progress of controllers and indicators of parameters like temperatures, controller settings and other technical values. These systems usually lack visual information about human manual interference. Fast changes in process are hard to watch. Simple operations like valve opening or closing or motor switching and operation times are often not recorded. In order to make it possible to trace such situations in the event of malfunctions or process deviations, a visual tool for the recording of process graphics, pictures and controller profiles was developed. The requirement defined with pilot plant users from TUM was to establish easily accessible historical pictures of the real process. This must be achieved including recording times of the product life cycle in order to support back-tracking of products according to DIN, EAN, EU and international standards. The system was set up in order to be easily accessible from the on-line MMI, with a resolution time of 1 second which is then played in fast motion mode. The recordings are performed via standard software on a common PC. The addition of recorded process situations and data is a helpful tool in optimizing process operations. It is also an ideal help in developing new process recipes and test runs. Furthermore it can be used for operator training.

P-114

Decentralized easy-to-use wastewater treatment plants for the future

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The motivation of the project is the worldwide growing demand for water and the existing restrictions in access and water quality, which will drastically increase in the future due to worldwide developments. Additionally, in many areas the transport of fresh and used water causes high logistic efforts and energy consumption. This is dealt with through the development of decentralized plants that can be installed in units like sky scrapers, universities or factory premises. These plants are designed to produce service water that can be used for flushing or irrigation. For this purpose the application of anaerobic technology suffices and, furthermore, leads to a lower amount of sludge while produced biogas can be used to save energy. Wastewater treatment is basically realized by a two step process, where hydrolysis and acidogenesis are carried out in one reactor, acetogenesis and methanogenesis in a second. In addition to this classic approach a third step takes care of the ammonium load of the processed water. A major disadvantage of anaerobic plants lies in their sensitivity to overloading which may cause process failure. The following start up of the plant needs, dependent on the size, several weeks up to a few months. In a former study it was shown that this failure can be avoided by appropriate automation, which is also necessary for another reason. The idea of a decentralized approach leads to a high number of plants, which requires a sufficient number of operators. As it is not probable that enough experts in wastewater treatment can be found for this purpose, the plant will be equipped with a sophisticated automation system, based upon cognitive hybrids. Artificial neural networks are designed to extract hidden information from the sensor data. This not only gives refined knowledge of the actual plant's state, it will also be used to replace expensive sensors with cheap and robust ones. A further part of the automation system is represented by fuzzy logic, which allows the integration of expert knowledge into the algorithms. With this approach experience and knowledge can implicitly be supplied to the operator of the plant. Consequently, it is intended to enable caretakers, house owners or other people with similar knowledge to operate the plants and handle all events during normal operation. To guarantee suitable response to more severe problems, a remote maintenance system will be developed that enables a control center to supervise the plant state and take appropriate action to restore normal plant operation.

P-115

New retrofit cleaning-in-place system for open fermentors

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Although open fermentation is critically discussed in the literature, this procedure continues to be used by many small and medium size breweries. Advantages include both the ability to remove the foam formed during fermentation and better visibility of the fermentation process. However, a significant disadvantage is that most open fermentors have no tool for automatic cleaning, which makes manual cleaning a necessity. Proper cleaning of fermentors is essential for the hygienic production of beer. Compared to manual cleaning, automatic cleaning provides higher reliability and economic advantages because of more efficient use of labor, water, cleaning supplies and energy. A standard method of cleaning-in-place (CIP) for open fermentors is the use of a spray ball fixed in the middle of a mobile cover, which is then lowered on top of the fermentor. In this contribution, we present an alternative method of CIP for open cylindroconical fermentors in which cleaning fluids are applied through a pipe close to the fermentor wall. The system can be used without a cover and is a retrofit system invented by Trumer Privatbrauerei Josef Sigl in Obertrum. In the investigated system, a liquid water film is generated at the top of the vertical tank wall. For the cleaning process, uniformity and stability of the film as well as the interaction between film and dirt play a crucial role. Theoretical considerations were assessed and initial experiments were conducted to determine relevant parameters and specifications for the construction of a lab scale CIP system. The resulting model, with a diameter of 0.5 m, was equipped with both cleaning systems. Artificial dirt based on yeast composition was used to gain reproducible staining. The cleaning progress was recorded with a camera and evaluated with software tools on the computer. Finally, a prototype of the system was evaluated in a 260 hl cylindroconical fermentor at the Sigl brewery. The effectiveness of the cleaning program was evaluated by ATP measurements using bioluminescence and by microbiological means with NBB-Boullion. The described system allows CIP of cylindroconical fermentors within 60 min using standard cleaning agents. System specifications, including flow rate, media velocity and system dimensions, had a clear influence on the effectiveness and reliability of the cleaning. In direct comparison to a standard CIP system, both systems demonstrate suitability for the hygienic challenges encountered in beer production. Additionally, a new system to model fermentation stains is presented along with a new optical evaluation procedure to monitor cleaning progress.

P-116

Evaluation of a new method for water deaeration

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Deaerated water is primarily used in breweries to help prevent oxygen pickup in filtered beer. It can be used in filter preparation, DE preparation for precoat and dosing, purging lines pre- and post-filtration, blending high gravity beer, as well as for purging lines from bright tanks to packaging. Traditional means of deaerating water range from the very basic, such as bubbling CO_2 through a tank of water, to the more sophisticated, such as vacuum chambers or stripping columns. Increased sophistication often provides excellent results and much lower O_2 content in the water than basic methods, but at a significantly higher cost. A new method of water deaeration was tested that is easy to install in an existing tank and promises to provide results comparable to more sophisticated systems at a much lower cost. This method is based upon use of a modified cleaning jet machine positioned in such a way inside the tank that it can be used to both circulate the water for deaeration as well as clean the tank in CIP. CO_2 is injected into the circulating water, resulting in displacement of air. Results are tracked using a portable dissolved oxygen meter, moving from a basic system to the new system.

P-117

Practical usages of electrolyzed water (alkaline and acidic), as an antimicrobial agent in the process of sterilization without the use of chemicals

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Alkalinic and acidic water generating systems have been in existence for 50 years; it is only during the past 2 years that various practical cleaning and antimicrobial control applications have been possible due to electrolyzed water patented technology via specific metallic anodes and cathodes used as arrays in combination with specificities controlled by algorithms that control the activity of each. The objective of this study was to evaluate the effectiveness of electrolyzed water produced in an electrolytic cell to inactivate pathogens. Previously, the effectiveness of electrolyzed water has been evaluated for inactivating pathogens, ie. *Escherichia coli*, *Salmonella enteritidis*, *MRSA*, and *Listeria monocytogenes*, obtaining a considerable reduction in logarithmic units of CFU in comparison with the initial population on surfaces in *in vitro* experiments. (1) Electrolyzed oxidizing (EO) water is one type of functional water and has been used primarily as a sanitizer. Major advantages of using EO water as an antimicrobial treatment are that the EO water is effective, the apparatus is easy to operate, and it is environmentally friendly due to the production of the disinfectant using only water; thus, there is no need for handling potentially dangerous chemicals. Two applications for the brewing and beverage industries are immediately recognized: 1) organic matter breakdown and microbial control within the process and packaging areas without the use of chemicals or steam to clean, sanitize or sterilize; 2) de-scaling and biofilm control in cooling towers, tunnel pasteurizers, bottle washers, cooling and warming tunnels. This paper will present fundamental advances in the technology, equipment application specifications and methods. References: 1. Y.C. Hung, Dept. of Food Science & Technology, Univ. of Georgia, Griffin, GA.

P-118

Systems for HG-brewing

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A new generation of water deaeration, carbonation, and blending systems will be described. These systems are in a modular and compact design. The highlight of the system is its very high accuracy for °Plato or %vol. by alcohol in the final beer. Another point is the perfect dosing and solving of CO_2 in the product. In addition, the different systems for water-deaeration, such as vacuum and hot and cold stripping systems, will be shown.

P-119

Brewery 2010: Technical and technological prospects

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Developing and implementing brewery plants that operate efficiently and effectively in the long-term, while producing optimum product quality together with a fast return on investments and also low life-cycle-costs, are necessary because of increasing of prices for raw materials, energy and transportation, as well as a worldwide shortage of water and the moral commitment to save resources. When we look at grain prices on the world market we see that in 2006 the average world market price for grain was 146 EUR/t and in 2007 the price rose to 215 EUR/t. The same situation we find for energy costs. In Europe the energy increase from 1992 to 2007 is about 11%, in North America 14%, and in South America 26%. In the past, the focus was mainly on saving investment costs, but margins are exhausted, so now there is a big challenge for brewing technology suppliers to reduce operational costs by a) new layout and architecture structures; b) new ways of water treatment (collecting rain water, reuse of process water and using in process sections; c) high efficiency extract in wort technology; d) energy saving in the wort boiling step; e) combination of heat and power area; f) reduction of peaks and energy using with, for example, energispar motor; g) no waste technology means reduced costs for waste disposal, CO_2 neutral emissions due to residuals used as biogenic fuel and hence benefits on emission trading equivalent to savings of fossil fuel; h) use direct ammonia cooling systems and buffer systems to reduce peak loads in the cold block area; i) and so on. This presentation gives an overview of possible solutions for technical equipment and design and process technology which is necessary to fulfill the high technical and economical demands for a brewery in 2010, which are water: <2.3 hl/hl; electrical power: <12 kwh/hl; thermal energy: <4.7 kw/hl.

P-120

Guarantee material compatibility in routing

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When you transfer beer or wort from one vessel to another, are you guaranteed that it will not be contaminated? Of the many routes a material could take, have you selected the best choice? Efficient routing of materials in a brewery is essential for good brewery productivity, but it can be confusing for operations, and one slip can ruin a lot of good product. This paper will consider the demands of routing materials in a brewery and will illustrate methods which help guide operations in making appropriate choices. We will show how to protect against undesired mixing of incompatible materials with a compatibility matrix and will show how you can guarantee no inadvertent mixing in both manual and automatic operational modes.

P-121

Improved plant cleanliness, productivity, and efficiency through the application of ozone-injected water in plant sanitation processes

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Ozone, the tri-atomic form of oxygen (O_3), is a gas that is formed when diatomic oxygen (O_2) is exposed to high voltage electric fields or UV radiation. Ozone is an unstable molecule due to the weak bonds holding the third oxygen atom, making ozone a naturally powerful oxidizing and disinfecting agent. Established commercial applications for ozone include municipal water treatment, groundwater remediation, electronics manufacturing, commercial laundry, as well as sanitation processes in the fresh produce packing, food processing, winery, beverage, and bottled water industries. Ozone can be applied in commercial breweries in water purification and wastewater processing as well as a variety of sanitation processes, including surface sanitization, clean-in-place (CIP) sanitation of tanks and piping, and bottle and cap rinsing during filling. Our analysis of these applications reveals significant potential for greater plant cleanliness and overall productivity and efficiency. Plant cleanliness is enhanced by the superior oxidizing and disinfecting capabilities of ozone-injected water. Our analysis of the implementation of a cold ozone CIP system in a large bottling plant demonstrated improved microbiological results, significant savings of chemicals and energy, and greater plant efficiency. After installation, ozone CIP was progressively adapted to nearly three-quarters of the typical CIP runs. Microbiological testing revealed the three-step ozone CIP process to be more effective than a 5-step hot detergent CIP process. Microbiological tests for ozone CIP were 97% negative versus 81% negative for hot CIP. Our analyses also indicate significant potential increases in plant efficiency and productivity through the effectiveness of ozone-injected water at lower temperatures than traditional CIP protocols. Cold ozone CIP saves energy and chemicals and reduces the brewery's carbon footprint. Annual energy and chemical cost savings were $72,000 and nearly $300,000, respectively. Plant productivity was increased by significant reductions in sanitation process times and the elimination of CIP temperature ramp-up periods. The CIP run time was reduced by two-thirds from three hours to one hour. This time savings yielded an increase in overall plant efficiency of 4.1%, which represents an additional production of six million cases of product per year.

P-122

Improvement of the mashing process by means of vibration sources in mash kettles

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Brewhouse work is continually improved by technical and technological developments. As a result of increasing raw material and energy prices, attempts have been made to optimize the yield and reduce evaporation during wort boiling. In these efforts, the quality of the wort still has top priority. During the mashing process, vibration sources now are used to intensify the technological procedures. The vibration generator basically is an electric unbalanced motor, which can produce frequencies in a range of 0–200 Hertz. Soluble oxygen in the mash, which is mainly added during mashing-in, can be reduced by the vibrations. This causes fewer oxidation processes, which improves, among others, the aging stability of the finished beer. Depending on the concentration of the first wort, there is an ideal frequency range to activate the mash to a resonance vibration, thereby enhancing substance transport of the malt contents from malt particles to the fluid phase of the mash. It has been proven that there are more enzymes dissolved from the raw material, which causes quicker and more intensive extract formation in the mash. The intensified substance transport of course effects a higher yield, which can be proven, for example, by the convertible extract in spent grains. Enzymatic activity is increased by vibrations in the mashing process. The mechanical energy input causes an increased motion in the enzymes and substrate. As a result, more enzymatic catalyses are possible for each period of the mashing program, which allows a reduction of mashing time. In addition to the two classic parameters, rest period and rest temperature, there now is a new possibility to affect wort quality by means of the brew master.

P-123

Environmentally friendly CIP methods and chemistries

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A summary of research conducted by Ecolab Inc.'s Global CIP Technology Group focused on development of more sustainable cleaning methods involving reduced dependence on strong caustic compositions. Laboratory results and field case studies will be reviewed that demonstrate improved cleaning of hard to remove brewhouse and fermentation vessel soils using novel chemistry and methods that reduce alkali, acid, and phosphate wastewater discharge to the environment.

P-124

Water/wastewater sustainability techniques for breweries

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The brewing process requires a significant quantity of high quality water and consumes substantial levels of energy. Water is becoming an increasingly scarce resource than an assumed widely available ingredient. Energy costs continue to escalate in all geographic areas. These factors can dramatically impact a brewery's operations and overall profitability. Implementing water conservation and reuse techniques can lead breweries to higher levels of sustainability and benefit operations and profitability. Water is the largest ingredient required in brewing. The quality of the water can impact the brewing process and the overall quality (taste, color, smell) of the product. The specific water quality parameters are determined individually by each brewery. Reduced water quality has forced additional water treatment steps and increased water costs. Breweries can make a significant impact on local regions by conserving water use. The first step in water conservation is to determine water usage throughout the entire brewery. Specific locations and metering methods will be outlined. General water use unit factors will also be presented. The brewing process generates several unique high strength wastes as by-products. Many of these waste streams can have beneficial reuse applications. Spent grains and spent yeast have nutritional value as feed supplements and can be integrated into composting operations. The process wastewater generated by breweries typically has a high concentration of biochemical oxygen demand (BOD) from the carbohydrates and protein used in brewing. Brewery wastewater typically has an elevated temperature. The combination of soluble BOD and warm temperature make brewery wastewater an ideal substrate for anaerobic treatment. The anaerobic treatment of wastewater biologically transforms soluble BOD into an alternative fuel source known as biogas. Methane rich biogas has many potential applications such as supplementing natural gas to boilers and dryers. Biogas can also be used to produce electricity and heat in internal combustion engines, microturbines, fuel cells, and stirling engines. Biogas can even be used in cooling applications with absorption chillers. Examples of biogas utilization installations in breweries will be presented. Disposal of wastewater even at pretreated qualities can be difficult. Many municipal sewer and treatment systems have hydraulic or organic loading bottlenecks. Several food and beverage plants have been forced into additional treatment of process wastewater for reuse within their facilities. The use of multistage membrane treatment can result in nearly pure water quality that is often more pure than domestic or well sources. The reuse of water in breweries would significantly reduce water demand and disposal needs. Examples of water reuse projects will be presented.

P-125

Advances in preparation and processing of food and brewery wastes for energy recovery

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In a joint research project supported by AiF (German Federation of Industrial Research Associations) the technology and process for generation of biogas was investigated. Two areas for intensifying the process were focused on. The use of fine comminution of brewery spent grains together with other wastes, like bran, from flour mills was investigated, and different milling systems were used to reduce the particle size. With a premilling step in a dispersion mill wet material could be reduced to less than 200 µm. Further milling and reduction of particles down to <50 µm were used to increase the surface of the ground material for better access of microbial decomposition. A pilot plant was modified in order to allow optimal process steps in various reaction vessels. A novel biological microflora was adapted to the prepared substrate in order to intensify the hydrolysis and fermentation of the lignocellulosic compounds. The goal was to achieve a good quality of biogas with high methane content. The control of reaction parameters leads to reduced dwell times and makes economical plant definitions. The presented technology reduces some possible bottlenecks in waste disposal and contributes to an ecologically compatible supply of energy.

P-126

The use of carbon dioxide in the brewing industry and the effects of, and prevention of, contaminants and impurities on final product

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A by-product of the fermentation of beer is the generation of carbon dioxide (CO_2). Larger scale breweries collect the carbon dioxide generated by the fermentation process and re-use it for various processes. Packaging is a primary user of this carbon dioxide, where it is used for purging of bottles, cans and kegs prior to filling, in addition to being used to create counter pressure during filling. Carbon dioxide collected from the fermentation process is contaminated and contains high levels of impurities, rendering it necessary to purify the carbon dioxide prior to use. The quality of carbon dioxide used is important as it can have significant and undesirable effects on characteristics and can affect taste, odor, appearance or general presentation of the product, resulting in negative consumer impact. This paper details both the impurities and potential extraneous contamination present and their effects on product quality in addition to discussing the typical carbon dioxide purification process. Furthermore, a solution is presented in the form of an in-line static adsorption system which can provide an additional level of quality security in the event of impurities or contamination still being present after the initial purification process.

P-127

Mash application of glucoamylases—Transglycosylation and the effect on attenuation

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Limitations in malt limit dextrinase (responsible for the hydrolysis of α(1,6) bonds in amylopectin) means that worts may contain significant amounts of limit dextrins, and the maximum fermentability in normal beers is typically <70% RDF (real degree of fermentation). Microbial glucoamylases are saccharifying enzymes that can hydrolyze both α(1,4) bonds and α(1,6) bonds in starch. These enzymes give the largest increase in fermentability, with values of up to 85% RDF, when added to an all malt mash. From a further analysis of the malt carbohydrate, and the residual carbohydrate in the beer, it is clear that further improvements in RDF are possible. Part of the reason for this is that glucoamylases can also catalyze transglycosylation and synthesis reactions in the presence of high concentrations of maltose and glucose, resulting in the formation of unfermentable isomalto-oligosaccharides such as isomaltose (α-D-glucopyranosyl-(1→6)-D-glucose). The extent to which this transglycosylation occurs depends on the glucoamylase and other factors. Biochemical characterization and laboratory scale all malt mashes were carried out at Danisco, and pilot brewing was performed at Hopfenveredlung St. Johann GmbH. Standard methods were used to analyze malt, extract, fermentability, spent grain starch and wort separation performance. Carbohydrate and alcohol concentrations were measured by different HPLC methods. As part of a preliminary screen we have characterized the ability of glucoamylases from different micro-organisms, and glucoamylases with specific mutations, to form isomaltose from starch, maltose and glucose. Subsequently we have gone on to characterize the effect of mash application of these different glucoamylases in terms of extract, wort sugar composition and fermentability (as measured by RDF). As noted previously, the mash application of glucoamylases has the effect of increasing RDF from, typically, 65 to 85% RDF, depending on enzyme dose rate and enzyme identity. Increases in fermentability were due to the hydrolysis of limit dextrins, and starch oligosaccharides in general, giving worts that are high in glucose concentration. However, limitations in fermentability were, in addition to general enzyme properties, directly related to the ability of the different enzymes to catalyze transglycosylation and synthesis reactions forming unfermentable isomalto-oligosaccharides. These isomalto-oligosaccharides were identified and quantified in both the worts and final beers.

P-128

Development of improved enzyme products for attenuation control and very high attenuated beers

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The development and production of very high attenuated beer (LowCarb beer) created the need for better enzymes. Standard glucoamylase products from *Aspergillus niger* is used at high dosages and extended mashing times, but are sometimes accompanied by filtration and hot brake stability problems in the brewhouse. Glucoamylases can also be applied in lower dosages to increase attenuation during fermentation, but with a complicated pasteurization process to inactivate the enzyme. Glucoamylase products from *A. niger* also contain an acid stable α-amylase as a minor component. This α-amylase hydrolyzes amylopectin to smaller dextrins than other α-amylases and has suitable pH and temperature activity profiles for wort production. The glucoamylase and α-amylase work in synergy, where a higher α-amylase to glucoamylase ratio gives a more efficient saccharification. An improved enzyme product (Attenuzyme) with such an increased ratio has been obtained through self-cloning of the amylase into a glucoamylase producing *A. niger* strain. The improved performance when applying this product in mashing for high attenuated beer has been demonstrated. Further improvement in attenuation performance can be obtained through optimization of the dextrin debranching enzyme activity. Dextrin debranching activity (hydrolysis of α-1,6-linkages) is present in malt (limit dextrinase), and it is known that exogenous added debranching enzymes (pullulanases) increase saccharification. Different pullulanases have been evaluated in 100% malt mashing in combination with the acid amylase enriched glucoamylase enzyme. The evaluation included temperature and pH profiles and attenuation performance in 100% malt mashing. A new triple-enzyme product (Attenuzyme Flex) was defined by adding the preferred pullulanase in an optimized ratio to the α-amylase enriched glucoamylase. The performance of the triple-enzyme blend was compared in mashing to a glucoamylase product and the α-amylase enriched glucoamylase for high attenuated beer. The triple-enzyme product is effective at lower dosages of glucoamylase, where the same saccharification performance can be obtained with less than 30% of the glucoamylase activity compared to a glucoamylase. The product can furthermore be used to reduce the mashing time as the same saccharification with equal amounts of glucoamylase can be obtained with a 50% reduction of the mashing time relative to the glucoamylase. Brewing experiments demonstrate that the triple enzyme product also eliminates or reduces filtration and hot brake problems. The triple enzyme product is also applicable to improving the attenuation of maltose wort as the attenuation can be increased 4% more than with a glucoamylase with less reduction in maltose content compared to the glucoamylase product.

P-129

α-Acetolactate in sake mash, assayed by novel LC/MS method, was influenced by inoculum size and fermentation temperature

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α-Acetolactate is the precursor of diacetyl, which is the main cause of an off-flavor in alcoholic beverages, "*tsuwari-ka*" in sake or "diacetyl flavor" in beer. To predict the level of diacetyl in the product, the assay of α-acetolactate in mash is necessary. This assay has been done by heating samples and quantification of the resulting diacetyl. To ensure complete stoichiometric conversion, enzymatic conversion also was employed instead of heating. But, for some samples, especially for sake mash, these assays cannot give adequate results. A throughput of HPLC was enhanced, so the labile α-acetolactate could be assayed by the HPLC (UPLC) equipped with a mass detector. A centrifuged mash sample, which was two-fold diluted and neutralized by buffer solution, was directly injected. One UPLC run took 8 minutes, and the detection limit was less than 0.01mg/l. Neutralized samples were stable enough for several hours at 4 degrees. α-Acetolactate in sake mash was assayed by this method. This might be the first reliable result for sake mash and showed some different production patterns as the fermentation condition, such as inoculum size or fermentation temperature, differed. In beer mash, the production of α-acetolactate was closely related to the uptake of amino acids by yeast. Unlike the beer fermentation, that of sake proceeded parallel to the enzymatic digestion of steamed rice, and amino acids were gradually supplied from steamed rice to sake mash. A change in fermentation condition causes changes in supply and uptake of amino acids and should result in the difference in α-acetolactate production observed.

P-130

Fermentation course prediction with weight analysis

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There are situations in which brewers would like to know how compatible their yeasts are with their wort and the conditions at which they would like to ferment. These can be R&D tasks concerning yeast viability and vitality, wort composition (for example mashing schedule or addition of fermentation enhancing products like zinc), tasks concerning fermentation conditions (temperature control, pitching and aeration rate) or, for example, the need to see a fermentation curve at a fermentability test. For these and many others purposes the weight analysis test designed by Savel in 1993 can be used. The test is based simply on periodic weighing of a vessel with fermenting wort and computer supported calculations of alcohol content, real and apparent extract or apparent degree of fermentation. The test was sensitive enough to recognize wort enhanced with 0.2 $mg \cdot L^{-1}$ of zinc or low-aerated wort from normal wort at fermentation temperatures of 10 and 20°C. The mathematical base of weight analysis, which is discussed, can also serve as a fermentation performance prediction test. This

is based on fast (high temperature) fermentation of pitched and aerated wort sampled from a brewery wort line. Since the course of brewery fermentation depends not only on yeast vitality, the great advance of such a test compared to yeast vitality based fermentation prediction tests is that weight analysis results depend on both yeasts condition and wort composition. Weight analysis mathematics can also help microbrewers and homebrewers to control their fermentations without expensive analyzers and the need to sample from their fermentation vessels.

P-131
Withdrawn

P-132
Improving fermentor utilization by using natural hop antifoams

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The composition of wort makes it susceptible to foaming during fermentation. This is controlled either by mechanical means or by the addition of an antifoam compound normally consisting of a suspension of silicone compounds. Brewers are often reluctant to use silicones, and a fraction from hops has been isolated which can suppress foam formation. Brewing trials with this material have shown that in addition to effective foam suppression, the utilization of the isoalpha acids is significantly improved, and some negative flavor characteristics, such as the formation of diketones, is suppressed. Minor changes in the aroma and flavor composition have also been identified and are presented in this work, together with suggested mechanisms of their formation or suppression.

P-133
Yeast lag phase tracking: A toolkit for fermentation performance prediction

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Consistency of fermentation duration is a key issue for the brewing industry, particularly for fermentations that use freshly propagated yeast. It is generally accepted that lag phase can contribute considerable variation to total fermentor residence time. Variability of lag phase duration can be attributed to several factors, including generation number of the yeast, batch to batch differences in wort composition, fermentor physical environment and rate of yeast dispersal within the fermentor. Lag phase may be defined as the time required to progress from pitching to initial bud emergence. In this presentation, predictive biomarkers of lag phase progression will be identified that permit variations in this parameter to be rapidly detected, including DNA synthesis by flow cytometry; the expression of *SPG1*, *CHS2* and *CHS3* by real time PCR; and fluorescent tagging of key cellular events using confocal microscopy. Together, these biomarkers constitute a toolkit for predictive fermentation performance analysis. The potential of this during laboratory (2 l) and full (3275 hl) scale fermentations will be demonstrated. It is suggested that this toolkit will permit the development of effective process control, enabling more consistent yeast performance after pitching.

P-134
The effect of varying dissolved oxygen levels in wort on yeast fermentation performance in craft breweries

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Proper levels of oxygen have proved a necessity for yeast during the early stages of wort fermentation, as it plays an integral role in promoting lipid synthesis for cell wall production. Without an adequate supply of this building block, yeast cells characteristically display low viability and poor performance in fermentation. Recommended levels of oxygenation are in the range of 8–10 ppm; however, many craft breweries depend on existing protocols that do not involve measurements and may not be optimal. An investigation is described here that explores the adequacy of current dissolved oxygen levels in craft breweries and whether this has any correlation with fermentation issues, such as long lag time and slow fermentations. The range of oxygenation levels with respect to their effects on fermentation speed and the variance in dissolved oxygen requirements between laboratory grown cultures and multiple generation brewery cultures are also addressed. The dissolved oxygen levels of wort from a small sampling of mid-sized craft breweries were compared to the same wort at a measured 10 ppm in lab-scale fermentation trials. A commercial ale yeast strain was used for all fermentations, and fermentation vessels were kept at a constant temperature in a glycol-controlled water bath. The study is designed to determine whether craft breweries are sufficiently oxygenating and the impacts of this on yeast performance and repitching and to provide a possible approach to improve fermentation success.

P-135
Can -omics help high gravity brewing?

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When process optimization and economic savings are the keys to a brewery's financial success, high gravity fermentation is an attractive approach. The challenges of high gravity fermentation are associated with a number of stressful conditions for yeast such as high osmotic pressure, less available free amino nitrogen, high ethanol levels at the end of the fermentations and, as result of glucose repression, risk of incomplete fermentation. Knowing the complexity of the problem, modern system biology tools can offer insight into the physiological state of brewer's yeast in high gravity fermentations. However, while trancriptome and metabolome analyses are routinely used in systems biology to study baker's yeast, they are still less popular for studying the brewer's yeast genome and its metabolism. Considering the polyploid nature of lager yeast and the complexity of beer fermentation there still remains some problems when applying the systems biology approach to brewer's yeast. A few case studies based on our own research are discussed in this presentation. We characterized, under different wort conditions, three popular lager beer yeast strains with different ethanol tolerances. The strains were characterized at an average gravity of 14°Plato and at high gravity—21° and 24°Plato. The higher gravities were achieved with the addition of glucose or maltose syrups to the basic wort. The fermentations with wort at 21°Plato were also compared with fermentations at 21°Plato supplemented with different nitrogen sources. In all fermentations, samples for both transcriptome and intra- and extracellular metabolome analysis were collected from early exponential and stationary phases of the fermentations. Metabolome and transcriptome analysis of the samples from the stationary phase, especially in the case of the less ethanol tolerant strain showed significant differences between the samples grown in glucose versus maltose supplemented wort. The main metabolites that contributed to this separation were central carbon intermediate metabolites and metabolites associated with the pyruvate and phosphenol pyruvate metabolism. Comparison between the 21°Plato fermentation supplemented with glucose versus maltose syrups showed significantly changed genes associated with amino acid metabolism, cell organization and stress response. When comparing the samples from the 21°Plato control fermentations versus the 21°Plato fermentation supplemented with different nitrogen sources, the analysis showed increased amino acid content for the yeast cells both intra- and extracellularly, improved fermentation performance and a more favorable flavor profile of the final beer. In conclusion, both metabolome and transcriptome analysis can be used as tools to determine the physiological state of brewer's yeast in high gravity beer fermentations and help us further improve its fermentation performance.

P-136

Influence of fermentation temperature and high-gravity brewing on the synthesis of yeast-derived volatile aroma compounds

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As far as consumers are concerned, the aroma and flavor of beer are among the main characteristics that determine its quality and value. The aroma of beer is a unique mixture of volatile compounds originating from the malt, hop and secondary products formed during fermentation. The aroma complexity dramatically increases during alcoholic fermentation as a result of the synthesis of important volatile compounds by *Saccharomyces* yeast species. The nature and amount of these compounds depend on multiple factors, such as wort composition, fermentation temperature and yeast strain. The aim of this study was to quantify the differences in the synthesis of yeast-derived volatile compounds resulting from the difference in fermentation temperature and high-gravity brewing conditions. Volatile compounds were quantified at different stages of the fermentation and compared to the expression of 12 genes involved in aroma biosynthetic pathways. The volatile compounds synthesized by beer yeasts include higher alcohols (marzipan and floral aromas), acetate esters and ethyl esters (fruity and floral aromas) and carbonyl compounds (buttery aromas) among others. Higher alcohols can be synthesized either from intermediates of sugar metabolism, through anabolic reactions, or from branched-chain amino acids, through a multistep catabolic reaction, the Ehrlich pathway. Ester compounds are produced by condensation of an alcohol and a coenzyme-A-activated acid (acyl-CoA). Hence, in *S. cerevisiae*, acetate esters result from the combination of acetyl-CoA with an alcohol, by the action of the alcohol acetyl transferases Atf1 and Atf2. Correspondingly, ethyl esters are generated from acyl-CoA and ethanol by the action of Eht1 and Eeb1. Diacetyl is another important compound in beer. The final concentration of diacetyl in beer depends on three factors, namely synthesis and excretion of α-acetolactate, the immediate precursor of diacetyl, conversion of this precursor into diacetyl, and removal of diacetyl by yeast. The capacity of yeast to synthesize these compounds varies between different yeast strains. Although their exact contribution is not completely clear, fermentation temperature and the use of high-gravity worts are additional variables that affect the final concentration of yeast-derived aroma compounds in beer. As the enzyme activity of Atf1 and Atf2 is the limiting factor for acetate ester production, like the activity of Bat1 and Bat2 for higher alcohol production, we investigated if there is a correlation between the biosynthesis of these compounds and the expression of the corresponding genes, especially when a higher temperature or higher wort density was applied. Taken together, our study reveals whether the expression level and activity of the biosynthetic enzymes could be prime targets for flavor modification by alteration of process parameters.

P-137

Refermentation of aged beers: A new technique to elucidate the contribution of flavor compounds to the aged flavor

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Several quality aspects of beer are subject to changes during storage. Alteration of the flavor profile in particular is of great concern to brewers as flavor is considered the main quality parameter. Due to the growing export and globalization of the market, this concern has been emphasized, and the need for controlling flavor stability has grown. Despite 30 to 40 years of research it is not yet clear which chemical reactions and the corresponding flavor compounds determine the aged flavor of beer. Formerly, (E)-2-nonenal was regarded as the main cause of sensory changes during aging, but now it is evident that a myriad of flavor compounds is responsible for the overall aged flavor. The formation of these compounds is the result of numerous chemical reactions, like oxidation of fatty acids and higher alcohols, Strecker degradation, aldol condensation, furanic ether formation, degradation of hop bitter acids, Maillard reactions, etherification, terpenoid oxidation, glycoside hydrolysis and synthesis of volatile esters. In this work, the effect of yeast on volatile flavor compounds, which are suspected to contribute to the aged flavor, was examined. Hence, beer was aged and subsequently refermented. The advantage of this technique is that only those volatile compounds which are relevant for the aged flavor are considered. At first, sensory analysis of 3 pilsner and 2 specialty beers was performed by an expert tasting panel. The intensity of the aged flavor was rated as a whole and for separate typical aged flavors. After aging, a very strong aged flavor was perceived in all the selected beers. This aged flavor (eg. cardboard, caramel, ribes) was reduced significantly, however, after refermentation. This indicates that yeast was able to reduce at least a part of the compounds responsible for these flavor notes. Volatile flavor compounds (fresh and aging indicators) were analyzed with headspace SPME GC-MS. A difference could be determined between carbonyl compounds that can be reduced by yeast and non-reducible carbonyl compounds. Other staling compounds and fresh flavor compounds (eg. esters) were either unaffected by yeast or were formed during the refermentation process. The observed decrease in the aged flavor could not be fully explained, however, by the analysis of the known staling compounds as flavor thresholds were mostly not exceeded after aging. Therefore, flavor thresholds of several carbonyl compounds were reconsidered, synergetic effects were studied and other volatile compounds that were not yet linked to flavor stability were examined.

P-138

Pitching technology and oxygen supply with regard to yeast physiology—Effects on fermentation performance and beer quality

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Effective yeast management optimization as a key position within the brewing process chain should include a minimization of starting time, aeration and oxidative stress of the wort. This provides a basis for obtaining more efficient fermentations and enhancing beer quality as well as colloidal and flavor stability. The oxygen supply during propagation and fermentation in particular in "*Drauflassverfahren*", which is subjected to brew cycles, is still not adapted to the requirements of the yeast. Especially during "*Drauflassverfahren*" the right oxygen supply and exact time are determining factors for yeast growth and fermentation power. Under brewing conditions oxygen in yeast metabolism is only required for unsaturated fatty acids and sterol biosynthesis. As a result of the Crabtree effect the citrate acid cycle is discontinued, and the acetyl-CoA formed by the pyruvate dehydrogenase leads to product repression because the yeast is not featured with carrier systems for acetyl-CoA through the mitochondrial membrane. In this case the PDH bypass is of vital importance for generated cytosolic acetyl-CoA as a basis for lipid biosynthesis. The PDH bypass includes the pyruvate decarboxylase, the acetaldehyde dehydrogenase and the acetyl-CoA synthetase. By measurement of the specific enzyme activities of this pathway two significant metabolism branching points (pyruvate and acetaldehyde) for alcoholic fermentation and biosynthesis were captured. The sugar concentration in pitched wort as well as the oxygen level excite yeast metabolism pathways. The Crabtree effect and the repression of maltosepermease and maltase by glucose are two important phenomena. A successful switch between glucose and maltose application is the basis for an uninterrupted fermentation. The combination of flow cytometric optical analysis of the DNA content, the measurement of certain enzyme activities and the determination of sugar concentration during propagation and fermentation starting time provided a deeper insight into yeast physiology, the reaction of yeast to wort parameters and oxygen supply. At the same time the effects of yeast qualities like fermen-

tation power were examined on beer quality and flavor stability. The results of propagation tests and variations of oxygen supply by "*Drauflassverfahren*" show the possibilities of optimizing pitching technology. Oxygen itself provides an opportunity to influence yeast physiology which increases fermentation power and beer quality. In addition to this, yeast physiology in combination with yeast technology is a key to reduce costs of cooling systems and increase fermentation capacity.

P-139

Effective use of yeast nutrients to improve yeast nutrition and fermentation performance

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Sufficient yeast nutrition is a crucial factor for yeast propagation, fermentation and the physiology of the yeast culture. In addition to sugars and a nitrogen source, the yeast requires minerals, trace elements, vitamins, sterols and fatty acids. In all-malt worts the availability of essential nutrients is largely dependent on the malt quality and the wort production process. In regard to this the nutrient supply is typically sufficient in the majority of cases. However, although previous reports have focused on zinc deficiency it is likely that worts could also be deficient in the number of other ions and nutrients. Worts produced with adjuncts or sugar supplements show a different composition, in some cases the nutrients are often incorrectly balanced, which can result in poor yeast growth and abnormal fermentation performance. To counteract such issues, technological approaches are possible. One solution is the addition of the deficient nutrients by supplementation with commercially available yeast nutrients. In this study the effect of nutrient supplementation on fermentation performance was assessed. Different nutrient mixtures, including commercially available supplements as well as novel nutrient compositions, were tested during pilot fermentations. All malt-worts and worts supplemented with maltose syrup were fermented in parallel batches. Fermentation profiles, the production of volatiles, yeast growth, yeast vitality and the resulting beers were analyzed in each instance. The data obtained indicated that supplementation with yeast nutrients enhanced fermentation performance. These results could even be observed in worts which would typically be deemed as having a sufficient nutrient supply. The effect was also observed to increase over the following yeast generations (serial repitching). Thus a deterioration of the yeast culture over several generations can be prevented by altering the nutrients available to the yeast. By comparing the different wort compositions, it could be observed that increasing the concentration of nutrients is more valuable when added to low nutrient worts. In addition beneficiary effects during serial repitching were also observed in high nutrient worts. It is suggested that a better supply of nutrients may reduce yeast stress under high gravity conditions resulting in the improved physiological condition of the yeast culture over several generations. Differences in fermentation performance could also be seen by comparing the various nutrients. The results depended on the precise composition of the nutrient. Supplementation with a single nutrient did not show a significant improvement in fermentation performance. Surprisingly the addition of zinc was not observed to influence yeast performance during fermentation. Thus our results indicate that adding a defined composition of nutrients is typically most effective in improving fermentation performance, yeast and beer quality.

P-140

Ocean beer

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Seaweeds are rich in natural bioactive compounds. In particular, seaweed polysaccharides such as agar are present in the cell walls of some red algae and are composed of agarose and agaropectin. Agar was easily extracted from red algae and widely used as food and gelling agents according to the historic records of more than a thousand years in China and Japan. In recent years, agaro-oligosaccharide structures and bioactivities, which are derived from red seaweed polysaccharide, have been widely investigated. Many beneficial health properties of agaro-oligosaccharides are attributed to their antioxidant activities such as scavenging free radicals and inhibiting lipid peroxidation in various chemical assays. In addition, agaro-oligosaccharides have demonstrated in vitro and in vivo hepatoprotective effects. In this study, a marine bacterium strain, YT, with agar-degrading ability was isolated from the seashore of Kaohsiung, Taiwan. The YT agar-degrading enzymes were used to digest red algae and produce water-soluble oligosaccharides with functional properties (antioxidant activities).The oligosaccharides were then employed as adjuncts and added to a wort made from desalted deep sea water. Since deep sea water processing requires advanced technology, only the U.S., Japan, Korea, Norway and Taiwan have been begun to obtain deep sea water from a depth of more 200 m below the surface of the ocean. After yeast fermentation, a novel type of beer, ocean beer, is produced.

P-141

Cross-flow membrane filtration for producing neutral malt base

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Cross-flow membrane filtration has gained wide acceptance as both a technically sound and commercially feasible approach to molecular-level separations across a wide variety of food, dairy, and beverage processing applications in addition to the broader, traditional water treatment market. Newer U.S. regulations around the alcohol source of ready-to-drink, flavored malt beverages (FMB) has prompted the search for cost-effective techniques for producing a clear, neutral malt base for later formulation into FMBs in large commercial quantities. Cross-flow membrane filtration offers several technical and commercial advantages, including compact and modular design, relatively lower operating costs, and lower environmental impact. The cross-flow membrane filtration technique will be compared and contrasted with other production methods from both a technical and commercial perspective.

P-142

Diatomaceous earth: Nature's nanotechnology

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Filtration with diatomaceous earth is well established, and the process rightfully takes it place as both the most cost-effective and the most tried and trusted technology for the filtration of many liquids, beer included. The scientific principles behind its performance are to this day little understood. Ever since its first application for beer filtration in the first part of the 20th century, the search has been on for "something better" and perhaps a less mysterious alternative. But to this day, few rival processes have presented themselves with the same degree of flexibility, reliability, low carbon footprint of operation and cost-effectiveness. The regular and highly structured microporosity of diatomaceous earth has only now started to be better understood and its functionality is under wider investigation, in areas such as electronic microchip technology and for its optical properties in coatings. Its worldwide abundance and unique structure has assured its place in our technologically complex future. The reasons for the flexibility in its use as a filter aid and the inexhaustible nature of diatomaceous earth are detailed in this paper, explaining why it remains the number one solution for filtration, even after nearly 100 years of service. The mechanisms of particle entrapment using diatomaceous earth and how they differ from alternative methods are detailed. A summary is given of

the worldwide deposits and continued availability of diatomaceous earth for the foreseeable future. The conclusion is that diatomaceous earth remains a sustainable resource ideally suited for its application as a filter aid, both now and well into the future.

P-143

Aspects of beer quality and extract recovery with modern yeast management

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Increasing costs for raw materials and energy are forcing brewers to look into opportunities for increasing extract yield without compromising quality. After fermentation yeast contains a significant amount of extract. This extract can be recovered and added back into the process by concentrating yeast with the modern cross flow technique in combination with controlled Dia filtration. This poster presents aspects of the process criteria which apply to recovery of the extract and related analysis of taste active substances during the process. The described technology for extract separation is based on a ceramic cross flow technology with specific control features. By controlling feed, cross flow velocity, permeate flow and pressure trends in the process and adjusting the process to target set points continuously at point of process results in an absolutely gentle treatment of the yeast during the overall process. Most important is the control of permeate flux and pressure in direct relation to the cross flow speed and feed rate. By using Dia filtration from a defined process point on, the yield of the process can be increased significantly. In addition to extract a certain quantity of taste active substances also are separated during the process. The type and quantity of substances were measured at different points of concentration during the process. Also the impact of Dia filtration under different process parameters was investigated. Finally the impact on taste stability was investigated in relation to the blending rate of the extract to original beer and the blending point. Results show that by applying a controlled process parameter to a yeast concentration process in combination with Dia filtration the process yielded by the brewing process can be increased without compromising quality up to a 5% blending rate. Blending takes place at final filtration. The ROI of the investment is typically <2 years for an industrial plant.

P-144

Beer stabilization in combination with cross-flow filtration

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With the ever increasing interest in cross flow, rather than DE beer filtration, the method of beer stabilization needs to be reviewed in order to achieve the full benefits of dosage-free beer filtration. A fully automated cross flow filter in continuous operation does not match well to a batch operated stabilization plant. Adding stabilization agents upstream can influence the cross flow filter in a negative way. This paper emphasizes the use of a dosage-free (no dust) beer stabilization system in combination with cross flow filtration. Both the filtration and stabilization can then be operated 24 h/7 days a week, all without dosing any powders. Due to its vessel-free design, the head and tail handling of beer/water is negligible. The O_2 uptake is by far lower than with the DE filter/stabilization agent dosage methods. The stabilization system operates independently of the filtration system. This enables brewers to select their filtration systems from any of the cross flow filtration manufacturers. So far five beer stabilization systems have been installed behind cross flow filtration systems installed by well known suppliers to the brewing industry. This paper shows the set up, analytical results, costs, benefits and draw backs (if any) of such combinations.

P-145

The influence of non-starch polysaccharides on the filterability of wort and beer

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Non-starch polysaccharides mainly β-glucan and arabinoxylan are known to influence wort as well as beer viscosity and filterability. Even if the total amount of water soluble arabinoxylan is higher than the amount of soluble β-glucan, the research done so far has been mainly focused on β-glucan. Several papers describe the behavior of β-glucan in the malting and brewing process, including studies on the influence of shear forces during the brewing process and possible gel formation, respectively. It was obvious that the first generations of exogenous enzymes to enhance the filterability of wort and beer were mainly focused on thermostable β-glucanases which are able to hydrolyze β-glucan released at mashing temperatures above 60°C when malt β-glucanases are already inactivated. Another reason for focusing on β-glucans was the relatively smooth ability to measure the total amount of higher molecular β-glucan (>10,000 Da) in wort and beer by staining with Calcofluor and fluorescence photometer detection. The measurement of xylans is rather elaborate. For this research a new straight-forward method to measure all high molecular (and therefore wort and beer viscosity impacting) non-starch polysaccharides has been implemented. The high molecular weight polysaccharides have been separated by ethanol precipitation, and the xylans have been measured by the determination of xylose after acidic hydrolysis. The results show mainly two important results. First, xylans do contribute to wort and beer viscosity, and their influence on lauter performance as well as on filterability is measurable in the laboratory as well as in industrial scale. Even if the malt is well modified there are measurable benefits in filtration performance (7%) and extract yield (0.8%) when hydrolyzing the residual high molecular weight non-starch polysaccharides. If the malt is inhomogeneous or undermodified, which is quite common when dealing with the barley quality of the 2006 and 2007 harvests, the residual xylans can play a very critical role in production constancy. Hydrolyzing only the β-glucans will improve the filterability, especially when brewing with undermodified malt higher amounts of residual xylans can provoke weak filtration performances (–20%) and gel formation (blocking of the filter). Secondly, successfully hydrolyzing xylans with exogenous enzymes depends on the right choice of xylanases. Only enzymes belonging to a special family of xylanases (so called family 10) which are specific to the water soluble xylans are able to reduce viscosity to the desired level. The results will show an overview of how β-glucan as well as xylans contribute to wort and beer viscosity and filterability. Their development was followed over the whole production process in lab scale as well as in industrial scale. In addition their behavior after intensive shearing in lab scale will be documented.

P-146

Novel backwash technology for improved cost efficiencies in beer filtration

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In order to achieve the necessary clarity in beer, it is critical that yeast, protein, carbohydrate particles and other visible and sub-visible particles be removed. Removal of these suspended particles is often accomplished by filter sheets that are assembled in plate and frame filters and are precoated with a filter aid such a diatomaceous earth. The plate and frame filter has been around for many decades and is a workhorse for breweries around the world. They have a great advantage in that they have minimum operating requirements; however, they do require significant labor and time for set up, tear down and cleaning. Utilizing a novel filter cartridge composed of sintered porous plastic, it is possible to utilize the same filter aids, thus achieving the same clarity as the plate and frame, but by using a simple backwash step essentially eliminate the need to tear down, and reassemble. The backwash step typically requires less than 1 minute from shut down to start up, saving significant costs in labor and lost production time. This unique polyethylene media can be chemically sanitized and cleaned allowing for multiple uses before it needs to be discarded, thus reducing acquisition, warehouse requirements and disposal costs. The study evaluates the use of several filter aids in conjunction with the sintered porous plastic cartridge filter, optimizing the precoat loading and determining backwash effectiveness.

P-147

An innovative regenerable filtration aid—The future of diatomaceous earth-free filtration

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Diatomaceous earth (DE) is a natural filtration aid used during the beer filtration process for decades. The current consumption of DE worldwide by the brewing industry is more than 180,000 tons. Disposal costs for used DE are an increasing part of total filtration costs, and so brewers are commonly interested in finding more economical methods. Crosspure® is a synthetic polymer for optimal filtration and stabilization in general. It is intended to use as a regenerable replacement of DE which is additionally capable to removing tannoids, flavanoids and other haze forming polyphenols from beer. Just like PVPP, Crosspure® can be regenerated in a combined regeneration and filtration system—a closed system comprising a dosing vessel, filter unit and CIP system. The whole process was developed on a candle filter from Filtrox AG. The losses arising from continuous dosage and the resulting regeneration process are below 1.0%. In contrast to powder-free filtration technology, which implicates a fundamental Capex, Crosspure® can be used in existing, slightly modified DE filter lines. In general, this new filtration aid has significant benefits in comparison with existing conventional products, primarily because it is regenerable, easy to use, synergistically balanced and last but not least environment friendly.

P-148

Precoat filtration with regenerable filter aid

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Precoat filtration still is the state-of-the-art filtration technology for beer, with thousands of filter lines in operation around the world. Despite discussions about health risks and disposal costs, DE (diatomaceous earth) is the state-of-the-art filter aid for precoat filtration. For at least 15 years experts in the brewing industry have been looking for filter aids, which could replace DE, so far without success. Over the last years BASF has developed a new, regenerable filter aid called Crosspure, which was thoroughly tested on an industrial scale Filtrox candle filter. For the first time there is now a technically and commercially attractive alternative to DE available, which can be used to replace DE.

P-149

Use of isomerized hop extract as a replacement for conventional hop extract and its influence on beer flavor

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Raw material procurement is currently an important factor for brewers. Not only malt, but also hops are available in limited supply. Brewers using conventional hop products do have limited possibilities to react to this situation by optimizing their hopping recipe. One option to work around the shortage of hops might be the use of isomerized hop products. IKE (isomerized kettle extract) is one of the products which could be used as a replacement for CO_2-hop extract. In addition to any financial benefits it is essential to preserve the sensory characteristics of beer. In order to get reliable data, extensive, commercial-scale brewing trials were recently carried out. Analyses of hop products, wort and beer samples were made. Bitter substances were quantified using specific (HPLC) and unspecific (UV-Spectro) methods. For the analyses of hop aroma components, especially the character impact compound linalool, a method using GC-FID was applied for wort and beer samples. Samples were taken at different stages of wort boiling. This made it possible to obtain exact information about the solubilization of α-/iso-α-acids and the evaporation of hop aroma substances during wort boiling. Beer samples were evaluated by a trained tasting panel. It was demonstrated that IKE is a suitable kettle hop product which combines reduced hopping costs without affecting a beer's sensory characteristics.

P-150

Making the most of your hops

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Hop utilization has always been an economic concern for the brewer, and improvements in hop use were seen as a way to reduce the cost of brewing beer. However, with the critical shortage of hops following the recent 2007 crop, and expectations of a seriously tight supply for the coming years, hop utilization has turned from an economic consideration into one of hop availability that threatens a brewer's ability to produce beer. With traditional kettle hopping methods using whole hops, hop pellets and pure resin hop extracts, there have always been means by which brewers could improve the bitter and aroma yield from their hops such as adjusting boil times, raising wort pH and lowering wort gravity. These brewing modifications provide only relatively minor incremental improvements in utilization and are limited in their effectiveness. In recent decades, the development of pre-isomerized hop products such as isomerized pellets and isomerized kettle extract, as well as technologically advanced post-fermentation products including isomerized α-acids extract (iso) and reduced or hydrogenated isomerized α-acids extracts (rho, tetra, hexa), have resulted in significant improvements in hop bitterness utilization, as well as provided a wide range of enhanced hops functionality. In addition to the development of these well-established bitter products, there has been exciting new research and product introductions in the area of highly efficient and effective post-fermentation products that provide late-hop and dry-hop aroma character to beer. These bitter and aroma innovations have provided optimal consistency and utilization of hops components; however, up to this point, the advantages of advanced hop products and methods of hops addition have been explained in detail individually but not in terms of a holistic approach. Presented here are effective strategies and examples for the use of various hop products and combinations thereof that can significantly improve hop bitter and aroma utilization for both existing beer brands and potential new beer development, thereby stretching the existing limited hop supply and optimizing hop usage for the future.

P-151

Citra—A new special aroma hop variety

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Citra was selected to become a new hop variety because of a special flavor and aroma that it imparts to beer that is hopped using the variety. Depending on the brewing process and the hopping rate, the flavors and aromas of beer hopped with Citra can range from grapefruit to lime, melon, gooseberry, and lychee fruit. The variety Citra originated from a cross between the female European noble aroma variety Hallertauer mittelfrueh and a male that was derived from the variety known as U.S. Tettnanger. Citra is 50% Hallertauer mittelfrueh, 25% U.S. Tettnanger and the remaining 25% is East Kent Golding, Bavarian, Brewers Gold and unknown. The α-acids content of Citra ranges between 10% and 12%, the β-acids content is between 3.0% and 4.0% and the cohumulone content is between 22% and 24%. The oil content ranges between 2.0% and 3.0%. Citra produces solid yellow-green hop cones that mature during the first week of September. Production acreage for Citra is expanding.

P-152

Thermal isomerization of cohumulone

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Thermal isomerization is a key step in converting α-acids into iso-α-acids during the kettle boiling process. In an effort to understand the nature of this reaction and to produce the products more efficiently, we studied this thermal isomerization of α-acids under more closely controlled conditions in the absence of acid, base, metal ion catalyst, oxygen, and light. Cohumulone was synthesized and thermally isomerized in a non-aqueous solution under dark and nitrogen environments. It was found that mainly *cis*-isocohumulone was produced by a non-concerted reaction, but very inefficiently. The stereochemical assignment of *cis*- and *tran*-isocohumulone was re-investigated using C-13 NMR.

P-153

Impact of drought stress on content of xanthohumol in hop cones

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In recent years the therapeutic effect of beer has been emphasized more and more above all else because of specific components in hop cones. Current pharmacological studies show positive aspects of heterocyclic polyphenols, of which the most well-known flavonoid is xanthohumol. The remarkable pharmacological potential of xanthohumol can be deduced from a multitude of scientific investigations. In this investigation the influence of drought stress on xanthohumol content in different varieties and new Slovene crossbreds was determined. The content of xanthohumol varied a lot during individual years and among varieties, and it increased strongly under drought stress conditions. The results show a significant impact of circumstances on xanthohumol content. The same variety can in different circumstances achieve different xanthohumol contents.

P-153a

Stabilities of the free acid and potassium salt concentrate forms of iso-α-acids and reduced iso-α-acids

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Commercially available, viscous concentrates of iso-α-acids ("Iso") and reduced iso-α-acids ("Rho", "Tetra" forms) are prepared by addition of concentrated solutions of potassium hydroxide to the free-acid forms of the corresponding normal or reduced iso-α-acids. These concentrates are shown to have excellent chemical stability, although the Iso-concentrate does undergo a similar loss of potency in a high temperature, forcing test to that of conventional isomerized extract solution that is normally sold at an iso-α-acids concentration of 30%. However, the relative instability of the Iso-concentrate may be of little practical significance, since its major degradation compounds are eluted by HPLC in the region of the iso-α-acids and may simply be isomeric, perhaps similarly bitter, forms of the iso-α-acids. By contrast, the free-acid forms of normal iso-α-acids are quite stable, yet the rho- and hexahydro free acids are relatively unstable, forming compounds with HPLC elution times (using EBC 7.9 mobile phase) similar to that of α-acids for Rho, and tetrahydro-α-acids for Hexa. These compounds are believed to be formed from the *trans* isomers of Rho and Hexa, respectively, and by analogy with α-acids, are likely to be poorly soluble in beer.

P-154

Compositional analysis of monomeric and oligomeric flavan-3-ols in barley varieties and corresponding malts

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This work describes the compositional analysis of the monomeric and oligomeric flavan-3-ols in barley varieties and their corresponding malts. Although the phenolic composition of different matrices has been described by several authors, the concomitant analysis of the barley and its corresponding malt is rare. Ten barley varieties (spring barley varieties grown in the same field trial—Kromeriz, Czech Republic) and their corresponding malts were studied. Amulet, Bojos, Jersey, Prestige, Malz, Merlin, Sebastian and Tolar were the representative malting barley varieties and KM 1910 and KM 2084 were experimental hull-less varieties. All barley samples were malted using the same standard malting conditions. After steeping and germination the kilning procedure occurred in six successive heating steps: 50°C for 12 h, 60°C for 1.5 h, 65°C for 1.5 h, 70°C for 1.5 h, 75°C for 1.5 h, and 80°C for 4 hither characterization of flavan-3-ols from barley and malt, described in this work, shows catechin and prodelphinidin B3 as the major monomeric and dimeric flavan-3-ol, respectively. The content of catechin significantly decreases during malting for all the varieties with the corresponding increase observed for epicatechin. On the other hand, the behavior of the oligomeric flavan-3-ols seems not to be consistent during malting, as reported by other authors. In addition, the extraction solvent was demonstrated to impact the individual and total phenolic contents, with 70% acetone showing the highest capacity for the extraction of phenolic compounds, mainly proanthocyanidins, either for barley or malt. Finally, the results reported here show that flavan-3-ols, and prodelphinidin B3 in particular, should be considered as the main contributor of the free radical scavenging activity of barley and malt compared with other phenolic compounds. To our knowledge, the significant positive correlation found here between the content of prodelphinidin B3 directly in barley and malt and antiradical activity has never been reported.

P-155

Innovative powders from malt extracts—New CO_2 spray technologies

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Malt extracts have a well established position in the beverage industry because of coloring and flavoring properties. Because production is done without any additives they are considered a pure food, and no declaration is necessary. Malt extracts are used preferably as a replacer for sugar-coloring agents or sugar syrups. Typical application areas are alcoholic beverages, non-alcoholic beverages and powdered instant products for beverages. Malt extracts are aqueous extracts from a traditionally well-known raw material containing nutritionally and physiologically valuable ingredients, malted grains. For production, malt is mixed with water, the mash is then separated into soluble and insoluble components by a lautering (filtration) step. The dissolved fraction, the so-called wort is concentrated by subsequent vacuum evaporation until a dry matter of usually 60–80% is achieved. Such concentrates often show unfavorable properties, which mainly result from their viscosity and cause difficulties in further process handling. In process technology it is useful to transfer viscous, hard to dose liquids into an easy to handle powder form. Therefore various technologies have been developed to transfer malt extracts into dry form. Here spray-drying, vacuum-band-drying or freeze-drying have to be mentioned. Besides high processing costs (e.g. freeze-drying), oxidative process conditions (e.g. spray drying) and the strong hygroscopicity of the resulting powders are the main disadvantages of these processes. In this work two innovative and patented methods in spray technology using supercritical carbon dioxide were investigated to make powders with improved handling properties from viscous malt extract. One is the so-called CPF technology (concentrated powder form), the other is called PGSS drying (particles from gas saturated solutions). Both processes utilize the properties of supercritical carbon dioxide, so that on the one hand very gentle process conditions were used and on the other hand high quality powders from malt extract with enhanced properties in procedural behavior, compared with the common malt powders, were obtained.

P-156

Pulverized wort for brewing compared to traditional products

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German beer is famous and popular worldwide. However German ingredients are not available all over the world and are difficult and expensive to transfer. Moreover the procedure according to the German purity law demands sufficient experience in mashing and appropriate raw materials. Today it is possible and common to apply malt extract, which is extracted from wort by two-stage vacuum evaporation, instead of pure malt. Unhopped, thickened wort normally contains up to 30% water. The use of drying procedures (spray drying, freeze drying) enables the production of malt extract powder as it is already known for hop and yeast products. The lower weight and enhanced microbial stability of these powders are the main advantages in transport costs, shelf life and handling compared with

liquid products. For the production of beer from malt extract, powder is diluted with water and adjusted to the desired gravity. In this work several different worts were brewed using four different malt products in order to compare the quality of the resulting beer. One beer was made in a traditional manner with pure malt, according to the German purity law. Another beer was made with conventional liquid malt extract. The other two beers were brewed with two innovative powders. One powder consists of silicic acid which is normally used as a filtration additive and malt extract. The second powder consists of pure malt extract produced by a special drying process using supercritical carbon dioxide. Brewing was performed with the same process parameters in the brewhouse, during fermentation and storage. In order to compare the different beers, a set of common analysis and sensory tests were made.

P-157

Characteristics of oxalate oxidase in the malt

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Although oxalate has long been recognized as a problem in beer, there have been few studies devoted to the understanding of factors that impact its levels in beer. As part of our investigations on this topic, we have located and begun to study an enzyme from malt that can remove oxalate, namely oxalate oxidase. Oxalate oxidase is located in the aleurone layer and increases in activity during malting. It has been purified by ion exchange and size-exclusion chromatography. The molecular weight of the enzyme is 58.500. It has an optimum pH of 4.0. The Km for oxalate is 0.1 mM and for oxygen is 0.46 mM. The enzyme is activated by zinc and flavan adenine dinucleotide.

P-158

New barley varieties and their suitability for malting and brewing process

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Barley variety has a great influence not only on agronomic properties such as yield, fertilization and resistance to diseases but also on quality characteristics, i.e. on suitability for malting and brewing. In the past, the varieties offered by breeders were not satisfactory in all cases. They often showed a disharmony between cytolytic and proteolytic enzymatic power which can cause problems in processing performance and beer quality (taste, foam, colloidal stability, flavor stability). In order to integrate all partners involved in the supply chain (breeders, farmers, maltsters and brewers) the Berliner Programm was established several years ago. The objectives were to improve the information flow between the partners, to get closer co-operation and thereby a reduction in time between breeding of new varieties, evaluation of their suitability for malting and brewing and finally to obtain acceptance in the market. The Technical University of Munich Weihenstephan supports this program by analyzing barley and malt via micro malting with samples of 1 kg and finally by pilot malting and brewing at the facilities of the Trial and Research Brewery Weihenstephan. In this equipment batches of 200 kg of barley can be malted under all required conditions as in modern plants. Then, these malts are processed up to finished beers. These facilities allow malting and brewing under totally reproducible conditions. All the intermediate and final products are analyzed according to their special needs. This system has proven its ability to produce malts and beers with outstanding quality, which are fully comparable to commercial brews. This paper shows the facilities of the plants and an overview about the technology used in malting and brewing of new barley varieties. The analytical results show how this support is important for the decision on whether a new variety could be accepted by the market.

P-159

Factors predicting malt extract: A statistical approach within a single barley cultivar

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The amount of extract a malting barley cultivar can produce in the brewhouse will always be of crucial economic importance, and malts with high extract are desired. While the extract level of a particular cultivar is influenced by genetics, environment and malting practice, it is anticipated that that if quality grain is selected and optimally malted, extract levels will not vary much within samples of the same cultivar. Nevertheless, differences are observed in commercial practice, and the objective of this study is to determine which factors are most important in determining extract within a narrow population using statistical analysis. Four barley samples of the six-rowed malting cultivar Tradition were selected for the current study. All were of acceptable quality for malting. A randomized complete block design using barley sample, kernel size, germination days, and malting type as independent variables was carried out to give a wide variation in extract. Using analysis of variance and stepwise regression, results showed that soluble protein contributed the major variation (79%) in extract under different modification levels. However, under the same modification level, barley protein, 1,000-kernel weight, and diastatic power explained the most (74.3%) variation in extract. The predicted extract equation takes the form of Extract = $89.3 - 1.64 \times Pr + 0.16 \times KW + 0.019 \times DP$.

P-160

Association mapping analysis of malting quality in western Canadian two-row barley

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Association mapping (AM) is a relatively new mapping method in the plant sciences that is ideally suited to analyze data derived from large unrelated groups of genotypes, such as elite breeding lines and varieties. Utilizing the large number of molecular polymorphisms present in such genotype groups allows an accurate determination of genetic loci controlling important traits, such as those associated with malting quality. A total of 92 genotypes from the 1994–2006 Western Canadian Co-operative Two-Row Barley trials was collected from participating breeding programs. These genotypes represent elite malting genotypes from eight different breeding programs evaluated over a 13 year period (1994–2006), including the currently most popular Canadian malting varieties AC Metcalfe, CDC Copeland, CDC Kendall and Harrington, and newer varieties such as Newdale, Calder, CDC Select and CDC Aurora Nijo. Analyzing elite malting genotypes should help identify genetic loci which underlie the subtle variations that differentiates premium malting varieties from good ones. Quality data collected on these lines includes α-amylase, β-glucan, diastatic power, soluble protein, fermentable extract and friability. DNA was extracted from all genotypes and sent for DArT (diversity array technology) whole genome genotyping at Triticarte Pty. Ltd., Yarralumla, Australia. DArT is a chip-based hybridization platform that can simultaneously survey a genotype for polymorphisms at several thousand marker loci. This technology identified 830 polymorphic markers across all 92 genotypes. A mixed-model approach was used for association analysis incorporating both kinship information (generated with the program SPAGeDi v. 1.2) and population structure (generated with the program Structure v. 2.2). Analysis of the population using both structure and un-weighted pair-group method (UPGMA) clustering (based on the dice similarity coefficient) identified four genotype sub-populations. Results will be presented for loci associated with the malting traits. Identifying loci in the barley genome associated with malting quality will assist in identifying candidate genes governing these traits and allow molecular marker-assisted selection (MMAS). MMAS would limit the time-consuming, labor-intensive process of micro-malting to later generations in the breeding program when there are fewer lines to evaluate. Reducing numbers at this breeding stage would also allow more intense and complete testing of only the most elite selections remaining near the end of the breeding process.

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Investigating malting quality in U.S. barley breeding germplasm using genome-wide association genetics

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Breeding barley to improve malting quality is challenging because malting quality is made up of numerous traits which exhibit complex inheritance and are costly to analyze on a large scale. Genetic studies have been conducted to map genes involved in malting quality so that molecular markers can be used to increase the scale and efficiency of breeding procedures. Unfortunately, most of these studies have involved wide genetic crosses and revealed information that is not directly relevant to breeding. In this study, we investigated traits important for malting quality on elite breeding germplasm to identify genes and markers that could be employed in marker assisted breeding. Ninety-six breeding lines from the University of Minnesota breeding program were grown in three locations in Minnesota in 2006, and the harvested grain was analyzed for malt extract, soluble/total protein, diastatic power, α-amylase, malt β-glucan and β-glucanase activity. Standard malting quality parameters were analyzed using ASBC standard methods at the USDA Cereal Crops Research Unit in Madison, WI. β-Glucanase activity was assayed using the Megazyme method at standard and elevated temperatures to estimate thermal stability. Highly significant ($p < 0.0001$) phenotypic variation is present within the 96 breeding lines for soluble/total protein, diastatic power, α-amylase activity, and malt β-glucan. This variation ranges from 35.4 to 52.5% for soluble/total protein, 100–189°ASBC for diastatic power, 54.4–90.6 20°DU for α-amylase activity and 38–379 ppm for malt β-glucan. Significant variation ($p < 0.0197$) also exists for malt extract, ranging from 76.3 to 85.3%. These breeding lines were also genotyped with a set of 1500 single nucleotide polymorphism (SNP) markers distributed across the genome. Within the Minnesota breeding lines, 382 SNPs are polymorphic and spaced an average of 3.47 cM apart. We are conducting genome-wide association mapping of these quality traits to identify quantitative trait loci and linked genetic markers that can be used in breeding. The current status of this analysis will be presented.

P-162

Effect of high temperature–high humidity treatment of germinated unkilned barley on malt quality and extract characteristics

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The effect of a high temperature–high humidity treatment (HT-HHT) of germinated unkilned barley on malt quality and extract characteristics was studied. Two samples of six-row barley were steeped to 42% moisture and germinated, with and without gibberellic acid, at 15°C for 5 days. The germinated barley was placed in a high humidity (75–80%) atmosphere maintained at 45, 55, and 65°C, respectively. For each temperature, treatments were carried out for 30, 60 and 90 min, respectively. At 45°C for 30–60 min, the malts developed high diastatic power and proteolytic activity. The high values for cold water extract and reducing sugars in the extracts indicated extensive amylolysis of starch granules during HT-HHT of the germinated barley at 55–65°C. The worts were light in color, with a pH of 5.3–5.8, and titratable acidity was in the range of 0.09–0.23%. A consistent increase in soluble nitrogen and Kolbach index was observed in the malts treated at 45–55°C for 30–90 min. Free α-amino nitrogen of the malts was in the desirable range of 120–150 mg L^{-1}. Therefore, HT-HHT can be useful for improving malt modification and wort characteristics and to shorten the germination time for malts from poor quality barley.

P-163

A novel homogeneous enzyme immunoassay for rapid on-site analysis of deoxynivalenol in grain

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A new on-site test for the mycotoxin deoxynivalenol (DON) was developed using a novel homogeneous enzyme immunoassay (HEIA) method. In this new DON test, the technology is designed for rapid, quantitative analysis in grain matrices using a manual microplate-based format. The test mechanism was described in detail, and its performance was compared with commercial enzyme linked immuno-sorbant assay (ELISA) test kits and other analytical methods, including HPLC and GC-MS. Grain extracts were prepared for analysis from barley, malted barley and wheat. The DON value was tested with the new HEIA test in a side-by-side comparison with two commercial ELISA kits (Veratox and EZ-Quant). Results from this study demonstrate that the accuracy and precision of the DON HEIA test is consistently equivalent to the corresponding ELISA systems. Accuracy and precision were shown to fall well within the official certification requirements of the US Department of Agriculture. The results of this study indicate that the new HEIA test can be effectively used for on-site DON testing and offers the additional advantages of speed and simplicity. Relevant to the brewing industry, there is a compelling need for simple, rapid and reliable methods of DON analysis at on-site locations within the grain supply chain, most notably where testing is performed in grain elevators at high volumes. Currently, manual ELISA tests service the majority of these sites. As heterogeneous systems, ELISA requires multiple wash steps and timed incubations, as well as an appreciable amount of time and skill to accomplish. In contrast, the new homogeneous system described here eliminates these and other steps, reducing the procedure to a simple "mix and measure" approach that can generate results in approximately 5 minutes with accuracy and precision comparable to ELISA.

P-164

Isolation and characterization of two xylanases from *Fusarium graminearum*

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Fusarium head blight (FHB) causes severe yield losses and crop quality reductions in wheat and barley. *Fusarium* contamination of malted barley is also associated with problems that plague the brewing industry: mycotoxin contamination and the potential for beer gushing. It has been proven that cell wall degrading enzymes produced by *Fusarium*, especially xylanases, are involved in FHB infection. Two xylanases from the predominant species, *F. graminearum* (teleomorph *Gibberella zeae*), were purified and characterized. *Fusarium* cultures were prepared using wheat bran as the carbon source. The two xylanases were initially separated by ion-exchange and were purified to 52- and 40-fold, individually through subsequent gel filtration, HPLC ion-exchange and HPLC hydrophobic interaction chromatography. The purity and the relative molecular weights of the xylanases were estimated by SDS-PAGE to be 20 and 40 KDa, respectively. The two xylanases were identified by trypsin digestion followed by LC-MS/MS as the gene products of FG03624 and FG06445. In the mass spectrometer, for the high molecular weight xylanase, FG06445, 84% of the sequence was observed, while for the low molecular weight xylanase, FG03624, 65% of the sequence was identified. The predicted isoelectric points, optimal temperature and optimal pH were IEP 9.2 and 8.5, 45°C and 50°C, and pH 5.5 and 6.0, respectively. FG03624 showed much higher stability (35°C, 1 h, 93% activity; 45°C, 1 h, 58% activity) than FG06445 (35°C, 1 h, 63% activity; 45°C, 1 h, 20% activity).

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The linking of microbial community analysis of barley and malt using terminal restriction fragment length polymorphism (T-RFLP) with malt quality

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The indigenous microbial communities of barley harbor a wide range of micro-organisms. Many intrinsic and extrinsic factors influence the type and extent of microbial colonization, of which climate (rainfall and its timing) is widely accepted to be the most important factor. This investigation determined the typical microbial composition and load of Australian malt (and barley) grown in different environments and areas (typically very dry

harvest conditions) benchmarked against malt produced internationally (i.e., North America, South Africa and Europe) using terminal restriction fragment length polymorphism (T-RFLP) with further characterization of dominant members by constructing clone libraries. T-RFLP is a rapid, sensitive, sequence-based technique for microbial diversity assessment. The technique uses PCR in which one or both of the two primers used are fluorescently labeled at the 5′ end and is used to amplify a selected region of genes encoding 16S rRNA for bacteria and the D1/D2 domain of the 28S rRNA for fungi (including yeasts) from an extract of total microbial community DNA. This knowledge of microbial diversity is being applied to the prediction and investigation of the likely beneficial and undesirable components of barley and malt, allowing further investigation focused on the practical impact of these components on malt quality, brewing process efficiency and beer quality. Of particular interest is the undesirable malt quality problem, premature yeast flocculation (PYF), that results in slow or incomplete fermentations. It is widely believed that PYF is the result of microbial contamination by one or more key microbial entities. Rather than attempting to identify the chemical component/s that cause PYF, our approach is to identify the causal or associated microbes. By comparing various PYF positive malt with normal malts, we anticipate the identification of the putative microbes that are linked to this malt quality problem. Overall, we expect that the understanding of malt and barley microbial population composition and load from this study will assist the malting and brewing industries in developing rapid predictive tests for PYF inducing factors and mycotoxin producing fungi.

P-166

Application of classic brewing technology for a new generation of non-alcoholic beverages

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Internationally, there is a significant pull for alcohol-free alternatives to beer. Thus, in many leading beer markets non-alcoholic beer has established itself successfully. Only just recently, a new category of beverage products originating from malt and making use of fermentation was introduced into the European market. Selection of malt quality in combination with a fine tuned process allows producers to obtain clear and stable malt bases. These bases are used in combination with flavors and sweeteners to give an alcohol-free beverage with a unique and revolutionary taste profile. Sensory profiling is used to define key taste descriptors. Together with a detailed consumer study in Germany, different product ideas are worked out and ranked for concept and taste preference. Statistically relevant results show that the term fermentation—besides the fact that consumers are not able to explain it—is positively associated and highly perceived as a natural process by the respondents.

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New technology for gluten-free conventional brewed beers

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Celiac disease is an auto-immune disease caused by certain proteins called prolamins (gluten) which are found in wheat and barley. The incidence rate of celiac disease in the Western population is approximately 1:300 and is the most common chronic intestinal disease. Gluten intolerance is not only a topic for celiac patients but more often for health-conscious people. Consequently celiac patients and gluten-sensitive people have to live on a gluten-free or gluten-restricted diet, and of course the consumption of all malt beer has to be avoided. As there is a demand for beer in these groups of patients, different beers from gluten-free raw materials (sorghum, rice) and pseudocereals (buckwheat) are found on the market. But these types of beers differ significantly in sensory impression. We can demonstrate a new technology that allows the gluten-free rendering of classically brewed all-malt beers using the enzyme transglutaminase. This enzyme catalyzes the specific agglomeration of gluten and similar protein fractions. The agglomerated gluten particles can be filtered, resulting in a bright, clear beer with a gluten-free status. There are different methods available for the analysis of gluten in foods, among them immunochemical diagnostic kits (sandwich-ELISA) are widely used. However, these test kits do not work properly in beer, as the brewing process by itself includes hydrolysis reactions of proteins which could not be detected by these systems. Many beers containing high amounts of gluten peptides were said to be gluten-free, which was a misleading conclusion. Therefore, a new competitive ELISA kit has been developed for the detection of gluten peptides in beer. The new technology, which is protected by intellectual property, enables the specific removal of gluten from conventional brewed beer. With the new competitive test, gluten peptides are no longer detectable, and gluten-free status can be declared. This procedure can be used for any type of beer. There are no limitations regarding the brewing process. Hazy products like cloudy wheat beer have not been possible up to now. The characteristic parameters of the beer will not be changed. The sensory impression will remain the same as there is no change in the flavor profile. There is no loss in foam stability and cling. National regulatory bodies should be consulted for specific declaration guidelines at an early stage of product development, and local celiac associations should be contacted as well. The technology provides new opportunities for the brewing and beverage industry in the fast-growing gluten-free market.

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Influence of variety and provenance on the arabinoxylan content of wheat

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Arabinoxylans (AX) are essential structural elements of cell walls and are a part of the dietary fiber complex in cereals. The chemical structure of arabinoxylan is based on a chain of linear β(1–4)-D-xylopyranose units, which can be substituted with α-L-arabinofuranose in the O-2 or the O-3 position or both. Arabinoxylans in the cell wall may be cross-linked by diferulic acid bridges and possibly other condensation products of ferulic acid which complicates their solubilization. The content of water-extractable arabinoxylan (WEAX) is believed to increase during the germination process, as the cell walls are being degraded. The solubility of arabinoxylan increases with a higher degree of arabinose substitution. Among plant carbohydrates, arabinoxylans are non-digestible ingredients, which are not degraded or absorbed in the stomach or in the small intestine and reach the colon intact. Here they are mostly fermented by the large bowel microflora to lactic acid and short chain fatty acids, which can be absorbed and metabolized by the host. The content of arabinoxylans in brewing raw materials is of particular interest, because on the one hand they lower the extract content and so may lead to a lower final attenuation degree and to a lower process yield if they are insoluble. On the other hand, especially the water-extractable arabinoxylans might be desired because of their prebiotic and thus health beneficial properties. In this work twenty different wheat cultivars form different mounting places and harvests have been analyzed with regard to their content of total and water-extractable arabinoxylan. The amount of total arabinoxylan varied between 5.4 and 6.6% dry matter in 2005 and 4.1 and 6.2% dry matter in 2006. The amount of water-extractable arabinoxylan was from 0.67 up to 0.85% dry matter in 2005 and reached values between 0.58 and 0.9% dry matter in 2006. These results show the necessity to differentiate between wheat for brewing purposes and arabinoxylan-rich wheat suitable for the production of functional malts and foods. Additionally it is necessary to analyze every harvested charge because no correlation between total or water-extractable arabinoxylan content and variety nor provenance could be found. An influence of weather conditions could not be shown in this work, but it cannot be excluded at present. For the determination of arabinoxylan contents an existing method was modified and adapted to the special needs of wholemeal wheat grist. These adjustments are also shown in the presented work.

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More studies on the applicability of the non-fermentable carbohydrate isomaltulose in beer and beer specialties, and their remarkable results

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PALATINOSE™ (isomaltulose), a sucrose isomer, is a new functional carbohydrate that provides prolonged energy in form of glucose. Unlike sucrose PALATINOSE™ is low glycemic, low insulinemic and cannot

not be metabolized by a wide range of micro-organisms. It was suspected that PALATINOSE™ cannot be converted by most lactic acid bacteria and other beer spoilage organisms but also common beer brewing yeasts. Between 2004 and 2007, PALATINOSE™ was tested to determine its suitability as an ingredient in beer and beer specialties in research conducted at VLB Berlin. In this study it was demonstrated that most common brewing yeasts do not ferment and that typical beer contaminants are unable to utilize PALATINOSE™. With respect to beer-mix products, this aspect could be particularly important. Therefore comprehensive tests were conducted on shandy type products. Standard pilsner type beer was mixed with lemonade, the lemonade part varying only in the type of sweetener used: sucrose, PALATINOSE™ or intense sweeteners. While the sucrose-sweetened lemonade spoiled quickly, spoilage occurred much more slowly in the drinks containing PALATINOSE™. Following these studies, the influence of PALATINOSE™ on the microbiological stability of beer and beer-mix products was further evaluated, using lower concentrations of PALATINOSE™, comparing combinations of sucrose and PALATINOSE™, sucrose or intense sweeteners. All beer-mix products exhibited a reduced alcohol content of 1.2–2.8%. In a previous study PALATINOSE™ showed a reducing effect on the formation of typical ageing substances in beer, e.g. 2-methyl-butanal. This could result in the prolonged freshness and flavor stability of the finished product. As this aspect can be of great importance for beer manufacturers, the potential influence of PALATINOSE™ on the formation of oxidation products was further investigated in alcohol reduced beer. Concentrations of 1% and 2% PALATINOSE™ were tested. Over 9 months, the beers were evaluated sensorially, and the degree of oxidation and decomposition was measured analytically using the MEBAK test for reductive capacity, and the BAX-value determination according to Methner and Kunz. The BAX value is mainly affected by beer constituents, e.g. metal ion concentration, pH-starting value, and probably other substances in beer, and can be used to describe the different characteristics of beers. In this presentation, new results on the evaluation of PALATINOSE™ and its impact on the microbiological stability of beer and beer-mix products, as well as its impact on the development of oxidation products, shall be presented.

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Controlling fills in the brewing industry: Does Hot water jetting make a difference?

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A “DOE” method of investigation was conducted to determine if hot water jetting vs. ambient water jetting was effective at controlling fills on a high-speed bottling line process. There is only anecdotal evidence that suggests that better fill control can be derived from hot water jetting. Optimizing the package filling step is of considerable interest because of regulatory compliance as well as the potential for beer loss. This particular study was performed on a bottle line which had hot water jetting capabilities, but routinely used ambient temperature jetter settings because of mineral deposits which buildup in the jetter nozzle when using hot water. Furthermore, several different products with varying alcohol strengths were bottled on this line, which prevented the comparison of data simply by using long term averages. The bottle line studied demonstrated good performance for fill height and fill volume, but did not have the capability to sample by valve. Therefore, a screening experiment was devised to select four adjacent valves that demonstrated reproducibility. The data from the valve screening experiment was used to define the appropriate sample size to avoid the possibility of Type II error. A randomized complete block design (RCBD) type of experiment was performed where each valve was a “block” tested at two different levels (jetter temperature). The experimental set up required controlling at least nine parameters which could influence the overall data and “confound” the results. The samples from each of the four valves were measured for fill height using an Akitek fill height measurement apparatus, and then gravimetrically assessed for fill volume using the appropriate conversion factors. The results were analyzed for ANOVA using the StatGraphics 5.0 statistical software package. The data indicate that there was no statistically significant difference in either fill height or fill volume for samples that were hot water jetted compared to those which were ambient water jetted. Furthermore, the data suggest that the RCBD method of experimental set-up where the valve to valve variability was blocked was prudent.

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Development of a hybrid system for automatic recognition of particulate foreign matter in filled food on the basis of multi-contact excitation

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The presence of foreign matter in containers filled with food represents an extremely significant problem for producers and bottlers as well as suppliers and trade in the relevant industrial branches. Apart from image damage, the risks that arise from product liability—especially damage to persons—and consequential recourse claims have to be considered, as well as possible refusal of retailers to sell the products. The presented project focuses upon solid particulate foreign matter that cannot be handled by the usual optical detection systems. Presented are especially those cases where pieces of broken glass, here with characteristic dimensions >1 mm, in a glass container represent a high risk of causing injuries to the consumer. Although the emphasis is put upon this specific application, the system can also be used for containers of arbitrary optical accessibility and various materials, e.g. PET bottles, and a multitude of foreign particle materials, including metal splinters. All of these cases are handled with a solution approach, where the diagnosis of the existence of a solid particulate foreign body in food that is sufficiently capable of flowing, e.g. beer, juices, yogurt, is carried out on the basis of the vibrational response of the system food-package-foreign body to mechanical excitation by means of a neuronumerical hybrid. This system consists of numerical simulations and artificial neural network (ANN). Before excitation the particle is positioned near the wall of the container by an accelerated movement. The registration of the contact between a foreign particle and the package is realized optically and by piezo principle. Assignment of the response signal into the classes “particle detected” or “no particle detected” is done by the ANN. Numerical simulations on one hand are used for training of the ANN by producing a sufficient amount of training data. On the other hand they build the basis in the design of the experimental process parameters by estimating the impact of the transport induced flow upon the behavior of the particle and by simulating the reaction of the particle to the induced oscillation of the wall of the package. An important goal is the integration of the system into existing filling equipment, taking into account limiting parameters, e.g. cycle times, and various methods of vibrational decoupling. Additionally, different acceleration and excitation parameters are systematically investigated. The presented work was conducted with cooperation between the Institute of Fluid Mechanics, the University Erlangen-Nuremberg, and the Fraunhofer Applications Center for Processing Machinery and Packaging Technology (Fraunhofer AVV).

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Neuro-numerical damage detection of bottle crates by means of spatiotemporal vibration analysis

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A reliable, durable and fully automated damage recognition system for bottle crates is indispensable in the food and beverage industry, in order to ensure product and working reliability as well as a smooth operational sequence in the logistics chain. Also the endangerment of the product and company image by a damaged product causing potential injury to the customer plays a crucial role in today’s harshly competitive free-market economy, basically governed by advertising and price. Additionally, reliable identification of defective packaging before refilling facilitates a substantial increase in efficiency for the packing plant and thus lowers operating costs extensively. Considering a transportation cycle of 400–500 million crates annually, damaged and/or aged bundles cause enormous problems. For these reasons a hybrid, consisting of numerical simulations (based on mechanical vibration impacts) and artificial neural networks (ANN) was

developed within a project titled "Automatic Selection of Returnable Goods for the Food and Beverage Industry by Neuro-numerics". In the present follow-up research project it is combined with image processing. This further development of the already existing damage recognition system is currently carried out by the Institute of Fluid Mechanics of the University Erlangen-Nuremberg and the Chair for Food Packaging Technology of the Technical University of Munich. By replacing the laser-vibrometer used in the forerunner project an enormous reduction in system costs can be expected. As a superior result, the mentioned project aims at the conception and conversion of a before-competitive but practical system equipped with modern digital real time technology that can be trained online and maintained from afar. In addition the new method contains several innovative aspects compared to already available damage detection systems. In contrast to other measurement techniques, e. g. at pre-defined points, spatiotemporal vibration visualization is used for damage recognition of mass-produced articles for the first time. This allows the detection of micro-cracks and hidden damage at arbitrary locations in crates that current systems cannot recognize. Furthermore, an excellent detection rate, combined with an extremely fast diagnosis, is an important target. The major advantage of the developed system is the fact that attainable innovations are not limited to the food and beverage industry. Their spectrum of use extends over all economic sectors that deal with the production and the quality control of packages. Furthermore, the achievable innovations are able to supply a substantial improvement in customer safety and operation reliability. All-in-all the desired results supply an extremely sustainable basis for the exploitation of the latent, technical-economical potential, spanning various classes of business.

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Driving value by increasing bottling efficiency—Data based automatic fault localization

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Bottling plant machines are designed to keep the central machine running. Nevertheless plant efficiency-reducing downtime can occur. Downtime is caused by failures of the main aggregate itself or because of a starvation or blockage through failures of other machines propagating along the line. Identifying the responsible machine is not trivial. Normally machines are connected with transporters with a buffer function. Because of this, the propagation of failures varies with the buffered bottles. To increase plant efficiency the machine causing the most plant downtime must be identified for maintenance and correction. To save money and exonerate the staff in the bottling line this identification should be automated. As a base for automatic fault localization, standardized data is needed. To assure this a standard for production data acquisition of bottling plants was developed in cooperation with the industries. Regarding the results of an international survey these standards are highly accepted and implemented in the brewing branch. Based on this data, different approaches were used. On the one hand an algorithm was developed, which is able to identify the machines causing the central aggregate's downtime as well as the machines which emptied or filled the buffers in an undesired manner. The algorithm is based on a tree-structure of the dependencies in the plant. The different branches describe the propagation of failures. The decision on which way to choose is made by an analysis of the machine operating states in calculated timeframes. On the other hand mathematical models of the components of a bottling plant were built. These models enable the usage of a so called model based diagnosis (MBD) engine which was developed at the MQM Group of TU Muenchen. The idea of MBD is to compare a model of the failure-free operation with observations from the system. If there exists a contradiction between observations and model a diagnosis of all possible faults is made. To narrow the failures down it is also possible to define models of the faulty behavior of the components. The advantage of this solution is that only component models have to be developed. With a given system structure an automated diagnosis can be generated by the generic diagnosis engine. Both approaches led to good results. Whereas the pure algorithmic solution shows very good results with partial responsibilities for downtimes, the MBD solution is more flexible. In the future it might be possible to use it for other technical tasks as well. Summarizing one can say that the automated diagnosis of bottling plants can be realized automatically. The different paradigms have their individual advantages and offer a great opportunity for extensions.

P-174

3-Step cold sanitation of fillers

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Typical 3-step hot Clean-In-Place (CIP) programs for filler sanitation require large amounts of energy to heat the water to 185°F and several hours to complete. Research conducted on fillers indicate that a significant portion of both the energy and time typically used with CIP fillers can be reduced by replacing the 185°F water rinse step with a cold oxidizing rinse. This reduction in time and energy allows for quicker changeover times between products and therefore increased operational efficiency while continuing to maintain the highest level of food safety and brand protection.

P-175

Practical applications for dry conveyor lubrication

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Dry conveyor lubrication is an area of recent innovation for the packaging hall. Conveyor lubrication without dilution water can lead to significant water savings and operational improvements. This paper is a follow up from our dry lube introduction given at the 2007 MBAA Annual Convention and will review practical applications for conveyor lubrication with regard to water consumption and use, operational improvements, safety and aesthetics.

P-176

Approach to easy opening for aluminum can ends

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Kirin has researched easy opening of can ends. According to previous research, it was demonstrated that the residual breaking force (the opening force) score and the gap between the tab tail and panel (the gap under tab) were primary factors for easy opening of can ends. Therefore opening force reduction and quantification of the relationship between the gap under the tab and the easiness of can end opening were researched in this study. With regard to opening force reduction, the appropriate can end model was given by FEM (finite element method) analysis to reduce opening force. FEM analysis demonstrated that the shape of the tongue hole and the score profile around the rivet are effective in reducing opening force without deterioration of can end performance. In addition, FEM analysis led not only to opening force reduction but also to quantification of the relationship between the gap under the tab and easiness of can end opening. The digital human model, which is based on FEM theory, was applied to quantify the relationship between the gap under the tab and easiness of can end opening. The digital human model proved that the width of the gap under the tab significantly affects the easiness of can end opening because the pulling force which fingertips can generate becomes larger when the gap under the tab becomes slightly larger. For example, a digital human model demonstrated that the pulling force becomes 40% higher when the gap under the tab becomes 0.5 mm (2/100 inch) larger. Finally, the test sample which was designed by FEM analysis was evaluated by sensory evaluation to confirm the validity of FEM analysis. Most subjects judged test samples as better than control samples (ordinary can ends) in regard to easy opening. As a result, the validity of FEM analysis was confirmed, and the appropriate can end profile was obtained. Kirin now is investigating how to put the appropriate can end model to practical use.

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Enhancements to the flavor stability of beer through reaction rate improvements in oxygen scavenging crown liner compounds

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Recent studies have illustrated the use of electronic spin resonance (ESR) spectroscopy as an indicator of natural antioxidant concentration and flavor stability in beer. It is also possible to extend this technique to the evaluation of active packaging materials and the their impact on beer stability over the course of the shelf life. In this study, an evaluation of three different crown liner materials was conducted, correlating oxygen control with sensory analysis and ESR measurement of the beer's endogenous antioxidant potential (EAP). As expected, beer packed under oxygen scavenging crown liners exhibited higher flavor stability through both measurement techniques, than that under non-scavenger controls. Furthermore, improvements to the oxygen reaction rates within the liners correlated with sensory and EAP improvements toward the latter half of the six-month shelf life. The oxidation of beer has been well attributed in the literature to the formation of unsaturated aldehydes, through several reaction mechanisms. Although the mechanisms differ in their reactive components, they all involve species formed from molecular oxygen, highlighting the importance of oxygen control both in the brewing process and the beer package. As the reaction rate of the oxygen scavenging crown liner is improved, it consumes oxygen at a rate that is more competitive with the natural uptake of oxygen in beer, and flavor stability over time is enhanced.

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Development of a new sensor to control bottle conveyors

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In modern bottling plants a variety of machines are working together. Each machine in the plant executes a specific function. To make the single machines work, bottles have to be transported from one machine to the next. For this conveyors are installed. The conveyors are commonly used as buffers as well. They have the functionality to compensate for the downtime of a single machine. There are two types of buffers: the so called anti-starve buffer supplies bottles to the next machine while the machine before it has a breakdown. The anti-block buffer absorbs bottles during the breakdown of the following machine. Integrating these buffer types in a bottling plant can reduce plant downtime caused by short downtimes of single machines. Following this strategy, it is important to control buffers correctly. Nowadays the filling level of buffers is detected by inductive or capacitive switches which are activated by the accumulating bottles. The disadvantage of this method is that the fill factor is determined only in steps. So the speed of the conveyor can be changed only stepwise. Simulation studies at the Chair of Food Packing Technology showed that it would be better to change the speed continuously. Following this the efficiency of the plant could increase up to 5%. For this concept of a continuous control a new sensor is needed, which is able to count grouped bottles. Two kinds of sensors have been developed at the Chair of Food Packing Technology. One is a combination of standard sensors with a PLC (programmable logic control), the other is based on a CMOS-camera (complementary metal oxide semiconductor), which takes pictures of the passing bottles. The analysis of the pictures is performed by a special processor unit based on a FPGA (field programmable gate array). This intelligent camera transmits the number of counted bottles to the PLC, which calculates the correct speed of the conveyors. First experiments with different sensor systems also showed other advantages of this kind of control: reduction of noise by the slower impact speed of the bottles; reduction of abrasion of the bottles (scuffing); higher utilization of the buffer area.

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Improved operating conditions and product quality through regular and effective pasteurizer cleanings (boilouts)

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Monumental improvements have been evidenced in the condition of operating pasteurizers over the last decade. Water treatment chemical feed and control has been vastly improved, and much capital has been spent to fine tune the control of water treatment chemistry. Quality assurance personnel are now acutely aware of the importance of maintaining hygienic conditions in and around the pasteurizer. As a result, biological slime formation has been drastically reduced. With this improvement, contaminants that were previously undetected now make up the bulk of the deposition. Conveyor lubricant residues, can lacquer overcoat and "necker lube", now represent a major portion of the foulants. Traditional boilout procedures have been designed to deal with biological slimes and are not as effective for these organic contaminants. The changing matrix of the deposits necessitated a change be made to the boilout procedure and chemistry. This paper details ChemTreat's experience conducting exhaustive laboratory and field trials in major breweries to finally develop an effective procedure to facilitate the removal of these more tenacious deposits. The work also documents the correlation of the improved "boilouts" and the reduction of chemical use and improvement in product package quality.

P-180

New dimensions in draft line hygiene efficiency

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Today's draft beer market is characterized by strong competition. When standing in front of a long bar of beer taps, mature customers decide not only on brand and lifestyle, but also on quality and taste! The decision for a "second order" is strongly influenced by "the refreshing factor": a fresh and good tasting draft beer, however, is based on draft line hygiene. If customers complain about poor beer quality, the defendant might wish "to have a look inside the line". Traditional line cleaning is carried out with classic cleaning chemicals, whereby the effectiveness of the cleaner is not evaluated or monitored. The decision for the contact time is mostly based on experience or estimates. Once a cleaning regime has been chosen, it is used (automatically or manually) for all draft installations without adoption to the individual hygienic situation in the bar or restaurant. However when looking closer, it becomes obvious that a cleaning time can only depend on the grade of pollution in the beer line. The dirtier the line, the more cleaning is needed. What methods are available to check the hygienic status of the inner surface of a beer line? The traditional check of rinsing water either detects the microbiological residues (classic MB-testing), or ATP containing substances in the water. In both cases water is supposed to be a good "cleaner" with good mobilization abilities, in order to solve deposits. When using swabs, only the accessible parts of the dispensing unit (lower end of the tap, tap head) can be checked. What remains is quite a bit of uncertainty. In contrast, a newly patented chemistry makes visible any organic residues in all areas of the inner pipe surface by means of Color Change technology. When an alkaline, oxidizing chemical based on PST comes in contact with oxidizable organic residues in beer lines, the original purple color changes its visible spectrum to green and further on to yellow: In addition, every color species corresponds exactly to a certain amount of organics. That's how a precise evaluation of the hygienic status of the (whole) dispensing system becomes available for the first time in the US. Chemically spoken, PST is a redox measurement system in an alkaline environment, where the immediate oxidation of residues is consequently shown in an infinite reduction of a color indicator. For an easy and objective color measurement the portable "verification-case" provides a quick read out by means of digital imaging. The real-time translation of the colors into readable numbers (such as passed/failed) is processed by a special software. Both, the high-tech chemistry and the ease of the application process will be demonstrated and experiences from leading European breweries will be presented.

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Latest standards in beer dispense

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This paper gives an overview of the technical and hygienic requirements in dispense systems in Germany. Until 2003, Germany had a law concerning the handling of dispense systems. In the course of streamlining of administration and the introduction of new European legislation, the old instructions were replaced by new regulations for this area of expertise. This problem exists not only for dispensing equipment. All fields of the industry have similar problems and have created advanced solutions. The laws represent the state of the art and best practices. For years, the DIN German Institute for Standardization has provides guidelines for better communication between the various parties. With the new legal situation, industrial standards have become more important. Therefore new and updated DIN standards were developed. All the requirements are exposed in these papers. Compared with the situation in other countries, German hygienic and safety regulation standards are very high. They are scientifically approved and certified. In Germany there are a number of DIN standards for dispense systems. These include technical requirements for fittings, couplings and screw threads as well as cleaning intervals. DIN standards are always kept updated and evaluated every 5 years. The author's institution carries out experimental tests regularly. The poster will show a synopsis of available German standards for dispense systems with the most important contents like dispensing equipment, kegs, fittings, hygienic design, materials, cleaning and corresponding results of the latest tests.

P-182

Non-returnable kegs bigger than 5 liters as a new way for beer export

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Exporting beer all over the world creates a big problem in the return of empties. When using barrels, breweries report deficits of up to 20% of the delivered containers. Growing investment costs for new barrels, high capital lockup, expensive return transport costs and other causes have led to new investigations in packaging beer in kegs for export. At the moment, there are four systems which have been introduced in the market or will launched in the near future. They all are non-returnable keg-systems. One system is a keg made of PET that is comparable with the production of PET-bottles. The system has standard fittings so it can be used in every conventional tap system. The second system is also a PET-container, but it has a bag inside. The advantage of this system is that the beer can be conveyed with pressure air. The third development is also a bag-inside-system. The technical innovation of this system is the reduction of the CO_2-content of the beer to 1 g/l. Before tapping the beer it is carbonated to the desired level. Looking like a steel keg, a one-way keg is the fourth disposable keg system. Its construction is built like a regular steel container but with the advantage that all materials are recyclable plastics. This paper compares technical and technological aspects, advantages and disadvantages. But all systems can save money when exporting beer.

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Investigation of the causes of PYF malt using a modified analytical method for the PYF potential

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Premature yeast flocculation (PYF) is a serious problem in the brewing industry because it causes low attenuation and results in an undesirable flavor in beer. The PYF phenomenon is induced by certain malts, which we call "PYF malts." To overcome the PYF malt issue, it is important to study in detail the relationship between the actual malting conditions and the PYF potential of the malt using reliable analytical methods. Our approach to the PYF issue is presented here in three stages. 1) Development of a reliable analytical method for PYF potential: there have been several analytical methods for estimating PYF potential. However, they frequently have problems with repeatability because of unstable fermentation. We found that putting in a boiling stone or similar object to release the CO_2 dissolved in the fermentation wort made small-scale fermentations stable. High repeatability was obtained on a 50-ml scale fermentation, and this allowed us to carry out a quantitative analysis. In addition, we succeeded in developing a 3-ml scale fermentation test in a spectrophotometer cuvette. This has advantages for research in which many samples need to be analyzed at the same time. 2) Investigation of the causes of PYF malt: our studies on the localization of PYF factors and micromalting tests using infected barley suggest that one of the causes might be infections from microorganisms on the barley. We screened more than thirty kinds of fungi from the PYF malt and investigated which of them caused PYF in the malt. Malts made from barleys infected with five strains of fungi were shown to be PYF positive, and the fungi were identified. In parallel with this, the influence of steeping conditions on PYF were investigated in a micromalting facility. Steeping with no aeration increased the PYF potential compared with aeration. 3) Monitoring of the PYF potential of all the malt samples shipped in 2006–2007: the PYF potential of every malt sample shipped was routinely analyzed using a 50-ml scale fermentation test. We will report the frequency of PYF malt found among them. We observed that PYF malts were produced continuously from certain malting plants, even when no PYF malt was found in other malting plants using barley from the same area. This suggests that in addition to the quality of the barley, the malting process is also an important factor.

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A new method to measure yeast flocculation activity in malt using lectin (concanavalin A) coated quartz crystal microbalance (QCM)

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Premature yeast flocculation (PYF) is the phenomenon whereby yeast flocculates prior to depletion of nutrients in the wort. Because this interferes, PYF causes low attenuation and results in an undesirable flavor in beer. PYF is caused by a substance called premature yeast flocculation-inducing factor (PYF-factor) in malt. It has been reported that PYF-factor is a high molecular weight polysaccharide. Nevertheless it is very important for brewers to measure yeast flocculation activity or to detect PYF activity in malt, only a fermentation test has been applied as the conventional method for a long time. A fermentation test is a test wherein the actual fermentation is performed with wort and yeast in a small scale taking into account yeast growth and gravity of the wort. A fermentation test is a kind of bio-assay, results from the fermentation test fluctuate and are influenced by the yeast condition and nutrients in wort. In order to dissolve this problem, many devices or protocols have been developed. However, a new method, which does not need yeast and fermentation, has never been reported. Therefore, we have developed a new method to measure yeast flocculation activity in malt without using a fermentation test. This method is very unique because yeast flocculation activity is measured using Concanavalin A coated quartz crystal microbalance. Concanavalin A is a lectin purified from Jack bean (*Canavalia ensiformis*). It has been reported that Concanavalin A has an affinity to PYF-factor. Quartz crystal microbalance (QCM) uses the nature of the quartz crystal which has a characteristic frequency that decreases regularly when something binds on the quartz crystal surface. We made a QCM sensor coated with Concanavalin A. Our method is composed of four steps: 1) the malt extract is prepared from the congress wort by the conventional HPLC technique, 2) a Concanavalin A coated QCM sensor and the malt extraction are mixed in the acid buffer, 3) the frequency of the QCM sensor changes (decreases) responding to the amount of the PYF-factor included in the malt extraction, and 4) the frequency change during 60 seconds is defined as the yeast flocculation activity in the original malt. As a result of the test with nine malt samples, the yeast flocculation activity measured by our method correlated well with that measured using a fermentation test. Using our method, we were able to measure the yeast flocculation activity or to detect PYF activity in malt without yeast and fermentation.

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The effect of hop harvest date on flavor stability of dry hopped beers

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Dry hopped beers have a distinct hoppy aroma and flavor. Hops is said to contribute to flavor stability in both ways, masking aroma effects can increase the flavor stability, but degradation of hop substances during beer storage can also have an adverse effect on flavor stability. These beers were brewed with Hallertauer Mittelfrueh hops picked at 5 different harvest times. The resulting beers were evaluated fresh and after storage of 3 and 6 months at different temperatures. The results of the sensory evaluation of this beers will be discussed.

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The making of a professional beer panelist

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Brewers around the world have been tasting beer and teaching people to taste beer for a long time. In the 1950s, Arthur D. Little started training brewers to use the flavor profile method of sensory analysis, which was already being used throughout the rest of the food industry, to evaluate beer flavor. Since then the methodology and training have evolved, and thousands of beer flavor panelists have been trained around the world. This presentation will take a close look at the training that is necessary to produce a professional beer flavor panelist today. The steps include sensory screening tests to select candidates, general training in sensory methods and terminology, and specific training to be able to objectively describe beer flavor and changes in beer flavor.

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The effect of hop harvest date on sensory characteristics of dry hopped beers

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This project investigated the influence of the hop picking date on the sensory attributes of a dry hopped lager. Hops of Hallertauer Mittelfrueh were picked at 5 different harvest times, from very early picking dates to very late picking dates at 4 different locations (hop gardens) in the Hallertau. A standard lager was brewed using the same hopping regime for each beer, including kettle and dry hopping. With extensive analytical and sensory analysis the differences in the beers are discussed. It was shown that not only the picking date has a significant influence on the sensory characteristics of the beers. These findings will help to determine the optimal picking date for certain hoppy characters of this hop aroma variety.

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Influence of non-volatile beer constituents on mouthfeel and body of beer

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Drinkability of beer is promoted by good harmony between the different beer ingredients and aroma compounds. In consequence body and mouthfeel are important factors for a high drinkability and harmonic beverage. When a food or beverage is placed in the mouth, the overall sensation as a result of the perception of taste, odor (aroma) and texture (mouthfeel) is defined as flavor (Woods 1998). 'In mouth' sensory properties of beers encompass multiple and interacting sensations, like sensations of acidity, sweetness, bitterness, retronasal aroma, perception (flavor), viscosity, warmth, and astringency. The impressions of a beer's smell, taste, body, carbonization taste and bitterness were evaluated by the sensory tasting of beer. The importance of beer-tasters achieving a common understanding of terms describing mouthfeel is important. For this reason the beer industry has a standardized terminology wheel of mouthfeel and taste terms (Meilgaard et al. 1979). Nevertheless by description of body or smoothness in a sensory evaluation, often the characterization of the attribute body or mouthfeel is not very specific. In addition non-volatile beer constituents, which are responsible for mouthfeel and body, are not sufficiently known. Components of the beer matrix like alcohol content, dextrin, pH (organic acids) and proteins have an influence on body and mouthfeel and for example hop polyphenols are often named in literature as contributing to mouthfeel. This presentation shows the influence of different substances of the beer matrix on mouthfeel and body. A human taste panel is trained to describe their sensations with standardized terminology and a uniform developed taste schema. The effect of different substances (polyphenols, proteins, ethanol, dextrins) for improving mouthfeel character and body in aqueous solution and beer were tested. Afterward different matrix compositions were performed, and the changes in mouthfeel and body caused by the varied matrices were evaluated.

P-189

Influence of non-volatile beer constituents on the bitter taste perception of iso-α-acids

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The hop derived bitter taste is one of the most important and most intrinsic properties of beer. The main bittering sources are the iso-α-acids and in the case of the use of downstream products derivates of the iso-α-acids, which are responsible for most of the perceived bitterness. In addition to the concentration of the bitter principles there are further factors influencing the perception of beer bitterness. Because of the complex mixture of ingredients beer can possibly mask some taste and flavor compounds. Masking is a widespread phenomenon and a typical effect in heterogeneous mixtures, like beer. There are different theories about the contribution of ethanol, dextrins and pH-value on the bitter taste perception of beer. The perception of bitterness and the harmony of all ingredients is assumed to have a great contribution on the drinkability of beer. Drinkability means a specific harmony of all antagonistic substances in the beverage. It is influenced by technological and non-technological (physiological) parameters. One of these technological parameters is the composition of the beverage. Changing the composition of a beverage has a great effect on the perception of bitterness. In our experiments we changed the composition of model solutions and of an unhopped lager. In aqueous model solutions dextrins had masking properties and increased the threshold of iso-α-acids. In an unhopped beer they showed the same pattern. Dextrins may act as masking agents in the beverage and are an antagonistic part of the bitter taste. Ethanol has for some tasters a bitter taste. This results in a decreased perception of bitter acids in the beer, so the thresholds increase. But it seems that the bitter taste of ethanol itself is reduced by other constituents in the beer. So we claim that ethanol, dextrins and the acidity of a beverage have a great impact on bitter taste perception. A harmonic composition of these factors is essential for beverages with a high drinkability.

P-190

Identification of aroma compounds associated with sourness

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Beer aroma is comprised of various compounds, the balance of which is very important to taste profile. Consumer research reveals that indications of sourness can decrease taste preferences. It is conceivable that the sour aroma in aroma components may be a contributing factor, separately from sour flavors represented by organic and inorganic acids. Depending on the concentration levels, this sour aroma may be characterized as odorant. To improve beer flavor requires that the aroma associated with this sour odor be controlled. In our research, we utilized GC-olfactometry in an attempt to identify compounds associated with sour odors in beer. As a result of our research, we were able to detect at least 10 components linked to sour odors, mainly odors associated with fruit, cheese or natto. Using GCMS, we were able to identify aliphatic acids, aliphatic acid esters, and higher

alcohols. For several of these compounds, we were able to confirm an identical aroma between a reference standard and our GC-olfactometry. These compounds, either individually or in combination, may contribute to sourness in beer.

P-191

Sensory detection thresholds of iso-α and tetra-hydro-iso-α-acids in lager beer evaluated by ASTM 1432

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Previous research in our lab has shown qualitative differences between iso-α-acids (Iso) and tetra-hydro-iso-α-acids (Tetra). Questions have been raised as to whether there are panelist-specific sensitivity differences between these two compounds. The objective of this study was to measure and compare the individual human taste thresholds and group thresholds of Iso and Tetra in lager beer using ASTM method 1432. Threshold values of Iso and Tetra have been published using ASTM method 679, which is the rapid method for determining group thresholds of added substances; however, ASTM method 1432 is currently the standard method for determining individual and group thresholds. In this study, 14 volunteers were trained in 3 1-hour sessions to familiarize the panelists with the samples and testing procedure. Six replications were completed during testing, in which each panelist was presented with a series of 6 3-alternative forced choice tests. All panelists wore nose plugs to eliminate olfactory influences. A sigmoidal response was fitted to each panelist's Iso or Tetra concentration versus correct choice data, and the detection threshold for each compound was determined as the concentration where the panelist correctly chose the dosed sample 66% of the time (50% above chance). Group thresholds were determined as the concentration on a rank probability plot where 50% of the panelists could not detect the compound 66% of the time. Confidence intervals (95%) were calculated for the group according to a rank-probability plot. The group-wise detection thresholds and 95% confidence intervals of thresholds for Iso and Tetra were 7.1 ppm (4.5–11.2 ppm) and 2.7 ppm (0.7–10.0 ppm), respectively.

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Ethical drinkability testing: A novel approach to measure preference without exceeding government guidelines

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As in any production industry, sales volume is a key success factor, and the brewing industry needs to understand what makes one product more drinkable than another. Drinkability may be defined as 'product preference after consuming a given quantity of the product'. If the preference changes during the drinking experience, then the product is deemed not to have a high drinkability. If the preference does not change then the product has retained its drinkability. Understanding why customers consume more of one drink compared to another is a complex issue. We would like to understand this process more clearly, but investigations are hampered by ethical issues. In order to explore the concept of preference in relation to beer, researchers will often use extended drinkability testing. However, traditional extended drinkability testing, whereby respondents are asked to consume relatively large quantities of beer, can raise concerns over ethics. Is it acceptable to ask respondents to drink in excess of their daily recommended number of alcohol units? Using an alternative and novel method of assessing drinkability developed at BRI, the desire to continue drinking was investigated together with how preference changes as beer is being consumed. The key factor for this novel method is that the quantities consumed within the drinkability session remain within ethical guidelines for moderate daily alcohol consumption and is therefore a more acceptable method for such studies. This method is a way of assessing a key sales parameter without upsetting public morals and can also explore reasons whereby why some consumers switch beer brands during a drinking session.

P-193

Sensory comparison of the same lager beer stabilized through two different techniques: Pasteurization and bottle conditioning

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The shelf life of beer is one of the major concern for brewers and, as is known, it is obtained through the pasteurization process. Nevertheless to preserve the "handmade" characteristics of a product, the shelf-life can be improved by bottle conditioning without heat treatment of beer. Industrial lager beers, generally characterized by low alcohol (between 4 and 5% by vol.) and extract content, after filtration, are pasteurized to obtain biological stability. Bottle conditioning is a technique generally used to produce top fermentation beers with an alcohol content higher than 6% by volume. To evaluate the effect of bottle conditioning on sensory quality of a lager, a bottom fermentation beer (pasteurized) has been compared to the same beer bottle conditioned with different yeasts. A pasteurized lager (sample P) and five bottle conditioned lagers (not pasteurized) with four yeast strains were tasted after 10 months. As is known after this time, sometimes even earlier, beers can show staling problems affecting shelf life. All tasted beers came from the same starting batch (SB) of filtered and not pasteurized lager; sample P was obtained from SB after pasteurization processing (21 PU, Pasteurization Units) while bottle conditioned beers were added with sucrose to have a final carbon dioxide content of 4.5 g/L and an amount of yeast to obtain a viability equal to 5×10^4 CFU/mL. All samples were stored at 20°C. The samples of bottle conditioned beer were kept at 23°C for the first month to allow the yeast to ferment the added sugar. A sensory test of all beers was carried out by a trained panel of 13 assessors; each sample were randomly tasted at the 10th month, and aroma and taste were evaluated considering several aspects using a rating test. Results showed that bottle conditioned beers were appreciated as much as pasteurized ones and, some of them, even more. Possibly due to its reducing power and oxygen scavenger effect, yeast acts as a protection against the off-flavor development mainly related to staling taste. Results showed that bottle conditioning can be an interesting and valid system even for bottom fermentation beer in order to obtain a stable and distinct product according the yeast strain used.

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Development and practical implementation of competency-based standards for professional beer tasters

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Objective evaluation of flavor is one of the most critical quality assurance tests carried out on beer in final pack and in-process. The reliability of such tests depends on the competence of the assessors and the number of assessors used to evaluate each sample. As with any high level skill, the aptitude and performance of the people carrying out the tests can vary greatly. Historically, beer tasters have been encouraged to develop their skills over a period of many years, building up experience in day-to-day tasting in the brewery. In some companies, however, an erroneous link has developed between taster status—as indicated by job title or number of years of service—and tasting ability. Unfortunately, taster status is an unreliable indicator of taster performance. To help address this issue and improve the skills of brewery tasters, we have developed and tested competency-based approaches to taster skills development and successfully applied them in partnership with a large number of breweries. We have used web-based technologies to collect information concerning the performance of about 4,000 professional beer tasters in more than 350 breweries over a period of five years. Our results have substantial geographic coverage, representing data from assessors in close to 100 countries. We have also collected and analyzed information from several hundred trainee tasters, acquired during intensive taster training courses. Our studies have shown that competency-based skills development programs provide an effective means of training professional beer tasters. Selection and screening of assessors prior to training, provided it is done in the right way, leads to a substantial improvement in training outcomes. Tasters who can demonstrate a high degree of competence in training and post-training testing also perform well in routine taste tests. Those who perform less well in

training and post-training testing also perform less well in routine taste tests.

P-195

A new approach to sensory evaluation

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Flavor is the significant criterion of evaluation in beer tasting, which depends on many primary and secondary causes. This includes desirable and un-desirable aromatic compounds, but also haptic and physical issues like viscosity CO_2-level and color. Taste as a subjective matter is valued individually quite different. Influences of process changes are difficult to evaluate. In order to get representative and fast results, a new sensory test was developed, which can be realized with reasonable resources, and takes statistical methods into account. The relative number of test persons who can detect a difference in change remains almost constant in a group and more or less independent from external influences. Individual errors follow statistic behavior and can be assessed if the number of tasters is big enough. The significance of results from a smaller group of experienced tasters in a sensory panel can be improved by increasing the number of test persons even if they are less experienced. The results of this work are based on differentiation trials with beers with different concentrations of benzaldehyde as a typical off-flavor aroma compound. In a group of inexperienced tasters, mainly young food technology students, qualified differentiation showed high conformance and significance. This test indicates that the method with the incorporation of statistical methods can be used efficiently for the evaluation of process changes and their effect on beer taste. The developed functional coherence can be applied for economic process optimization.

P-196

New highly aromatic products and distillates from smoked malt—Flavors and compounds

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The production of beers from smoked malt can vary in a wide range of smoked malt additions. Based on Pilsner malt, different percentages of Bamberg Rauch malt were added to achieve different characteristics of smoked beer flavors. Two different fermentation procedures with top and bottom-fermenting yeasts were investigated with respect to the profile of aromatic compounds. The main target was to optimize fermentation conditions like temperatures. All beers were analyzed and tested sensorially. In a second trial series the products were distilled in order to produce distillate products which can be used as flavorings. The process of distilling was performed in a column under various numbers of trays and flow rates and other process parameters. The resulting distillates were characterized by sensory and chromatographic methods. These can be used for the flavoring of innovative alcoholic beverages. The main goal of the trials is to optimize the process to get distillates with a well balanced aroma profile between smoke and malt notes.

P-197

Structures and properties of flavanoids involved in beer color instability

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Acetone/water-soluble polyphenolic fractions (70/30, v/v) of three lager beers from the same batch, differently stabilized before bottling in glass or PET bottles, were monitored by NP-HPLC-ESI(–)-MS/MS over a one-year period of storage at 20°C. In parallel, beer color was monitored by the EBC assay. The evolution of color was similar in the silica gel-filtered beer to that in identically bottled and stored PVPP-treated samples, despite the high flavanoid dimers content of the former. On the other hand, color evolved more rapidly in the PET bottle, suggesting a key role of oxygen. (+)-Catechin emerged as the precursor of less polar products characterized by a yellow-brown color. MS/MS enabled us to identify them as issued from the oxidation and intramolecular additions of dehydrodicatechin B4. Similar structures were found in aged beers spiked with (+)-catechin. Their stability and antioxidant activity were investigated. Beer storage in the absence of oxygen and at low temperature is recommended to minimize the synthesis of such pigments.

P-198

Proline-specific protease eliminates the requirement for a long cold stabilization step, saving substantial energy costs and reducing the environmental impact of sub-zero cooling

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(1) DSM

Beer is normally chill-proofed and subsequently stabilized by encouraging the precipitation of the so-called haze-active proteins with some polyphenols. This is achieved by chilling the beer post-fermentation to very low temperatures. The amount of precipitate depends on the temperature (mostly sub-zero) and the length of time (between 2 and 14 days is normal). The precipitate is then removed during normal beer filtration. However this is an incomplete process. Unprecipitated haze-active proteins and polyphenols remain soluble in the beer in varying amounts. These are removed, at least in part, by the use of chemical absorbents such as PVPP and silica gels. Haze-active proteins are rich in the imino acid proline. The addition of a proline-specific protease into the cooled wort at the beginning of fermentation is now established in many breweries. This enzyme cleaves the carboxy side of the imino acid proline thereby rendering the haze active protein incapable of forming large light-scattering complexes. The resultant beer is extremely stable. As the beer is effectively stabilized prior to the so-called cold stabilization step a series of experiments were designed to confirm, or not, the need for such cold processing treatment. A series of pilot plant (20 Hl) trials at iFBM in France were performed to establish whether the length of time at 0°C could be reduced with beers treated with the enzyme. Against a control beer treated with 40 g/Hl of PVPP after 10 days storage at 0°C the enzyme treated beer achieved the same results in 5 days. Further experiments were designed to show the effect of temperature on this process. A 'cold' temperature of +7°C was chosen as this is the temperature that many breweries use to package their beer. Control beers were treated with either PVPP or silica hydrogel and kept either at 0°C or +7°C for 1 day or 5 days. Various predictive shelf-life tests showed that the enzyme treated beers were stabilized, whereas the others were not. Real time storage tests at 20°C, with haze measured and visually assessed at 0°C, showed conclusively that the enzyme treated beers were perfectly stable after 7 months (evaluation on-going). Furthermore foam was hardly affected and sensory analyses were extremely acceptable. A sophisticated calculator has been developed to quantify the cost benefits. This can be adapted for each individual brewery. Studies were commissioned to show the environmental impact of this reduced energy demand and will be presented in detail.

P-199

The influence of the Fenton- and Haber-Weiss-reaction system on haze formation in stabilized beer

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During the last 40 years various working groups have described generally accepted models for haze formation mechanisms in beer. The interactions between polyphenols and proteins have been identified as the main reaction system. Based on this cognition the brewers utilize PVPP and silica gel to stabilize beer. Nevertheless chill haze or permanent haze formation can be observed in beer after a certain storage time. It is also well known that the presence of oxygen, higher temperatures, light, metallic ions and mechanical influences accelerate haze formation during storage, but the responsible reaction mechanism could not be determined satisfactorily up to now. Also the described approach according to an oxidation reaction which activates the polyphenols by generating ortho-chinons, which are able to react with other beer ingredients, is not able to explain haze formation in stabilized beer completely. Our investigations on detached haze by solid measurements using ESR at 77 K have approved ESR signals in

haze, which cannot be found in filtrate. The different ESR-signals are caused by stabilized organic radicals and ions like Fe^{3+}. These results indicate an interrelation with the Fenton reaction system, resulting in iron-(III)-ions and hydroxyethyl radicals. The application of several analytical methods (ICP-OES, ESR, gel electrophoresis) helped to characterize the composition of chill haze and permanent haze during storage. Based on the additional comparison of the development of the endogenous antioxidative potential (EAP) and haze formation during shelf life, an important coherence in the haze formation of stabilized beer could be observed. The analytical methods have clearly demonstrated that oxidative processes are the major cause for colloidal haze formation in stabilized beer. On the basis of the former postulated haze theories, a mechanism was mapped out, in which the reaction products of the Fenton and Haber-Weiss-reaction system in beer play a central role in the formation of haze during the beer aging. After consumption of the EAP, the reactive hydroxyl radicals and secondary radicals are generated by the catalysis of iron and copper ions. At the same time the formation of iron-(III) and copper-(I)-ions, as well as oxidation of beer ingredients and formation of stabilized organic radicals occurs. Due to the complex formation among oxidized polyphenol-protein-complexes and iron-(III) as well as copper-(I)-ions the development of chill haze can be observed. During the progress of beer aging the oxidized iron-polyphenol-protein-complexes, which include the stable organic radicals, react with each other by attendance of radical reactions and formation of covalent bonds. This process describes the conversion of chill haze to permanent haze. Based on the results the effectiveness of influencing factors on haze formation in stabilized beer can be better understood, and the arrangements to increase colloidal beer stability can be optimized.

P-200

Colloidal stability—The effect of excess stabilization

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Preservation of colloidal stability in bottom fermented and filtered beers can be regarded as one of the biggest challenges breweries have to meet in the current beer markets, which exhibit an ever increasing tendency toward globalization combined with rising consumer-expectancy of the clarity and quality of beer. One focal point of the current research is the improvement of the predictability of haze formation before filtration and stabilization to enable more specific beer stabilization and prevent excess stabilization. This in turn would lead to reduced costs for stabilization agents and the preservation of health-relevant substances such as polyphenols. The aim of this study is to highlight the effect of excess stabilization on the composition and quality of the resulting beers. In this context unstabilized beer has been compared to PVPP-stabilized (50 g/hl) beer and to double stabilized beer (PVPP 50 g/hl, Xerogel 100 g/hl), each beer deriving from the same batch (Pilsner type). Analyses included monitoring of the phenolic spectrum and protein fractions as well as measurement of the reducing power, foam stability and colloidal stability. PVPP stabilization resulted in an obvious decrease in total polyphenols, flavanoids and haze relevant flavan-3-ols (measured by HPLC) but did not influence the concentrations of phenolic acids. Stabilization with silica gel induced a significant decrease in tannin-precipitable proteins; the reduction of total nitrogen was quite low. The measurement of the reducing power, using two electrochemical methods, brought out a significant deterioration of antioxidative capacity stabilizing with PVPP compared to the unstabilized beer. Foam stability was slightly worse after stabilizing with silica gel. The force test (0°C/40°C) in the unstabilized beer showed an increase in haze of 2 EBC already after 2 warm days; the stabilized samples can be regarded as excessive stabilized, showing an increase in haze lower than 0.2 EBC even after 16 warm days. It has been shown that stabilization should be done in a more specific and selective way to produce higher quality beer combined with lower costs.

P-201

The effects of proline-specific endoprotease (PSEP) treatments on foam quality in beer made from various malt varieties

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Both foam stability and clarity are definitive indicators of beer quality. The perfect commercial beer has a good head and is "brilliant"—in other words free from any haze. This image is problematic as there is a complex interrelationship between foam stability and haze, so that alterations or interventional treatment in one can affect the other. The proteins involved in both foam and haze are conventionally known to be fragments of hordeins and are relatively rich in proline. Haze is formed when polyphenols and hordein fragments form complexes large enough to deflect light, thus making a beer appear cloudy. New forms of haze treatment that specifically target proline-rich haze proteins have been developed recently. Proline-specific endoproteases (PSEP) are enzymes that hydrolyze proline-rich protein sequences, neutralizing the protein's haze-forming potential. Hordeins, however, have also been found to be both foam-promoting and foam-reducing. A recent study by Evans et al (*J. Am. Soc. Brew. Chem.,* 2008, 66(1):1-10) showed that treatment of beer with PSEP, as judged by the Rudin test, could either slightly improve or reduce foam stability while having little impact on beer lacing. This investigation extends these conclusions by applying the industry standard NIBEM foam stability test. The NIBEM test requires packaged, carbonated beer in order to assess the influence of PSEP on foam quality. A method for small-scale production of packaged, carbonated beer was applied successfully and an additional trial was conducted. Using the NIBEM analysis, the trial compared the effect on foam quality of hopping with isomerized, against hydrogenated, hop extracts. Increasing the levels of hydrogenated and isomerized hop extract resulted in substantially higher levels of foam stability (Rudin, NIBEM) and lacing (lacing index test). In all the tests, the results showed that hydrogenated hop extract was superior to isomerized for foam stability and lacing. According to the Coomassie blue binding and PRM total beer protein assays the addition of PSEP does affect the levels of haze-active and foam-active proteins. This investigation confirmed that varieties such as Araplies, Gairdner and Sloop have slightly improved Rudin foam stabilities while Schooner has slightly reduced. Foam stability as measured by the NIBEM test, however, was slightly reduced (~10 sec) with the addition of PSEP for all four varieties. This investigation agrees with earlier research that there are hordein fragments that are both foam stabilizing and destabilizing. The extent to which these foam active proteins also promote haze has yet to be established. Hordein banding patterns are heritable characteristics of barley varieties, so the accumulated information from the above assays may allow the selection of barley varieties that contain hordein species that are both more foam-promoting and less haze-active. PSEP products could aid these brewing and research objectives and prove beneficial to the brewing industry.

P-202

The influence of dark specialty malts on beer flavor stability

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Beer aging remains a hot topic in beer related science. In particular, the role of dark specialty malts in beer flavor stability generates contradictory evidence. Dark specialty malts are used during the production of several specialty beer types and are responsible for the color and typical flavor of the beers. Although several researchers state that dark specialty malts provide antioxidants, favorable for flavor stability, pro-oxidant capacity is also found. Color malt is dried in a kiln at higher curing temperatures then pale malt. During the production of caramel malt on the other hand, a two step drying procedure in a malt roaster is applied. Due to these differences in the production process, the chemical composition of both dark specialty malt types differs, although the malt color is similar. In this work the role of caramel and color malt in beer flavor stability was studied. Therefore two 16°P amber beers of 20 EBC were brewed using, respectively, 40 EBC caramel malt and 43 EBC color malt in a 5-hl pilot scale brewery. In order to find a correlation between beer flavor stability and the dark specialty malt used, the concentrations of different stale markers were monitored in fresh and aged beers using headspace solid phase microextraction, coupled with gas chromatography-mass spectrometry. Both beers were aged at 40°C for 2 and 4 weeks before gas chromatographic analysis. The concentrations of lipid oxidation markers were significantly higher in the aged beer made with color malt, although the concentration of the staling indicator in the fresh beer was lower than in its caramel malt beer counterpart. Other monitored staling compounds such as the Strecker aldehydes 3-methylbutanal, 2-methylbutanal and phenylacetaldehyde were

also found in higher concentrations in aged color malt beer. The Maillard reaction indicator furfural and β-damascenone followed the same trend. By contrast, furfuryl ethylether was higher in the caramel malt beer. The decrease of fresh beer indicators such as isoamyl acetate and ethyl caproate after aging was dramatic in color malt beer compared to caramel malt beer. Staling compounds were more abundant in the beer made with color malt. Therefore, the choice of a certain dark specialty malt type can have a major impact on the flavor stability of the corresponding beers.

P-203

Anaerobic and aerobic beer ageing

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Typical antioxidants found in beer, such as reductones or polyphenols, undergo degradation in beer during ageing. Antioxidants can be converted into highly reactive species which are able to react with natural beer compounds. These reactions are followed by the change of the typical beer attributes. The ageing mechanism is basically irreversible degradation of beer compounds. The presence of oxygen can accelerate and add some new features to the mechanism. The strongest reactive species can be generated during beer oxidation by the air, which has been illustrated in many literature sources. There is also an analogy between oxygen and Strecker type oxidation agents obtained from polyphenols and reductones. The exclusion of oxygen from the beer and headspace of the package were expected to stop beer ageing, but it has never been observed. The aim of this work is to find the basic mechanism of beer ageing and explain the relationship between anaerobic and aerobic ageing. Model solutions containing reductones as well as oxidized polyphenols were aged in the presence of metal catalysts. Natural caramelization products used in this work contained reductones and colored compounds similar to them which are created during the brewing process. The reactions were strongly accelerated in tap water compared to deionized water. This observation agrees with brewing practice because the composition of brewing water has a key influence on the attributes of the beer. The caramelization products can undergo oxidation reduction reactions in both anaerobic and aerobic conditions. There is some similarity between the fate of oxidized polyphenols and caramelization products during beer production and ageing. Oxidized polyphenols can also be created by heating of natural polyphenols and they can undergo degradation under anaerobic/aerobic conditions. Both groups of these compounds represent typical oxidation reduction and acidobasic indicators showing reversible or irreversible color changes. These changes can be studied by differential spectroscopy during heating or photolysis. Differential spectroscopy has been proved as a useful tool to recognize the subtle changes in beer even in tens of minutes after beer packaging. Model solutions of caramelization products and oxidized polyphenols were prepared by heating in the presence or absence of air, and their changes corresponded to the basic brewing operations such as brewing, fermentation, lagering and beer ageing after packaging. Another experimental approach in which beer was oxidized with the help of various oxidation agents (ODA [oxidative destruction analysis]) was studied. The customer orientated attributes such as beer color, haze, foam stability and flavor were measured during ageing. Beer was recognized to be a complex oxidation reduction system with slow electron exchange accelerated by light, temperature and oxidation agents which undergo partially reversible and irreversible reactions.

P-204

Withdrawn

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5S – A systematic approach to improving brewery operations

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“Cleanliness might be next to Godliness”, but 5S is a smart brewery’s best operational strategy. Breweries use a vast array of materials, machines and people to produce quality beer. Keeping your brewery clean and organized is necessary for the consistent production of world-class products, coworker safety, the reduction of production costs, and the maintenance of your brewery’s appearance. Your brewery is a reflection of your brand and the message you send your customers. The degree of cleanliness and neatness inside and outside your facility can enhance or detract from your identity in the marketplace. The Japanese manufacturing industry implemented concepts to maintain a clean and organized workplace in the 1950s using a system of steps each named with a word beginning with the letter ‘S’. 5S is now a major component of all world-class manufacturing and lean manufacturing systems. This system maintains a clutter-free workplace, with tools and materials made easily accessible, standardized cleaning practices and routine follow-up to ensure required tasks are accomplished. 5S implementation has been shown to result in a safer, more productive and more appealing workplace which can produce higher quality products. Although widely taught, few are able to realize this successfully. The concepts included in a 5S system, the importance of having a vision, the need for support from your leadership, ideas for the successful implementation of these concepts from a regional brewery and the resulting business benefits will all be discussed. A step-by-step process will be presented, typical challenges and setbacks will be shared, and ways to measure your progress while keeping people motivated will be presented. Details of how one regional brewer approaches this will be offered with their accomplishments to date. Both internal equipment and facility cleaning will be included. We will also show how this is a continuous process and business practice rather than a project.

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Developing the next breed of brewers in the 21st century

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The playing field of the brewing industry has changed drastically over the millennia with multiple technological advancements and, more recently, the globalization of the industry. The modern brewing company needs to develop and train the next generation of brewers so they may enter the industry at an accelerated pace and continue the life-long learning process from there on. Miller Brewing Company has developed a training framework to give new talent a strong foundation in the industry and is currently completing its second year of training. The training takes the young brewer through two phases: the first phase is an extended introduction to the industry and all the operations of the plant where the trainee can delve into projects while learning about the area. The second phase allows the trainee to dive deeper into their functional area in a role that floats between improving process operations, managing operations, and other miscellaneous functions, all with the primary objective of absorbing as much information as possible. The trainee’s performance is evaluated at regular intervals by all levels of the company’s operations staff culminating in a final evaluation at the end of the program. After the final evaluation, the trainee is then placed in an operations management position. The program framework in a sense embraces the old proverb that, “it takes a village to raise a child.” This presentation is a first-hand account of the program, projects and expectations faced while in the program and beyond.

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Regional characteristics-based brewery factory restructuring and its benefits

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We, Orion Breweries, Ltd., restructured our workplace based on our midterm management plan with a view to increasing the functionality as an organization and reducing costs. The renewal of the control system to monitor the production process has been also executed effectively with “the integration of brewing department and power-related department” in mind. The result showed that we were successful in reducing the workforce required in both departments from 45 to 17 persons. Moreover, we were able to enhance our engineering performance by making effective use of the staff who were freed from former tasks. Specifically, a debug laboratory was created in the engineering department to test and develop our original software programs, which is conducive to lower facility costs and a shorter construction period. The amount of money saved in six months was 15 million yen (from April to September 2007). Though it cannot be estimated in figures, the greatest benefit of this restructuring is that it made it possible to monitor and control both the energy (utility)

process, which was in charge of the power-related department, and brewing quality, which was in charge of the brewing department, together in the same place. This mutual monitoring system enables us to react to accidents expeditiously and prevents troubles. Another merit lies in the fact that it has improved the skill and motivation of our staff. The "restructuring" we have accomplished is not the last step but the first step. We have a few more challenges for the future. One is that all staff members obtain the abilities to do mutual monitoring in every section. In addition, by improving the abilities and skills of individual members and increasing the range of work they can do, some can afford to be involved in work other than routine, and "improvement of work environments" will be continued and expanded. Our main office and factory are in Okinawa island, located in the south of Japan, which has a different climate and culture from those of mainland Japan. In Okinawa, we have worked under the concept of "*Yuimaru*", which is a unique dialect meaning "working in cooperation". With this spirit peculiar to Okinawa, we intend to make the best use of the effect of our restructuring and advance toward our project.

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An investigation of three different yeast propagation methods and their effects on yeast health and the finished beer

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This investigation focuses on propagating a commonly available ale yeast strain by three different yeast propagation methods: a modified Carlsberg flask, a microbrewery's current 3 bbl propagation system, and the Frings aeration system. During the investigation cell counts, dissolved oxygen, pH, glycogen, and vitality/viability were tracked during the propagation stages and used to determine whether any creates differences in the yeasts between the systems. All three systems had varying results. The biggest difference that appeared between the systems was the cell counts which varied significantly. The Frings aeration unit produced cell counts of 50–75 million cell/ml more then the other two methods. This investigation focused on the differences between the systems and how they affect yeast performance, yeast characteristics, storage ability, and flavor in a finished beer. The propagated yeasts were then used to produce sample beers that were tested by GC/FID and a sensory panel to determine whether any analytical and sensory differences could be detected between the samples. Samples from each propagation method were stored and tested at monthly intervals to determine whether any differences appeared in the yeast characteristics, or final beer flavor over time.

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Serial repitching of dried lager yeast

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Although there is a diverse range of applications for active dried yeast (ADY) within the brewing industry, one of its major functions is as a replacement for freshly propagated yeast slurry. Recent reports have suggested that employing brewers ADY may lead to fermentation inconsistencies such as poor flavor production and aberrant flocculation. However, analysis of the fermentation performance of ADY typically involves a comparison between dried yeast and a brewing yeast culture which has already been used for several serial repitchings, rather than a freshly propagated slurry. It is widely accepted that freshly propagated yeast is not perfect in terms of its fermentation performance and that the subsequent beer is often blended to eliminate any negative characteristics. Consequently, comparing the fermentation characteristics of beer produced with serially repitched yeast and ADY may be misleading. Here we evaluate the fermentation performance of wet and dried lager yeast over the course of serial repitching to investigate the differences between fresh and repitched cultures for each type of yeast. In addition, the capacity of yeast populations to adopt fermentation characteristics typical of the strain during serial repitching was determined. Each fermentation was monitored for a variety of characteristics, including sugar utilization and production of flavor compounds, higher alcohols and esters. In addition, yeast cultures were monitored for viability and the presence of petite mutants, flocculent variants and changes to the genome structure. The latter was assessed by analyzing chromosome length polymorphisms and the stability of delta regions flanking yeast transposons. The data presented here indicate that brewers' ADY can be used for serial repitching without any long term adverse affects in terms of genetic stability or fermentation performance.

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Differential transcription of genes involved in nutrient uptake during full-scale brewery fermentation

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Changes in the nutrient composition of wort during brewery fermentation can directly affect yeast metabolism and growth and influence the flavor profile of the final product. The complexity of wort compositional change is matched by the complexity of the yeast cell's response to these changes. In this study, changes in the lager yeast transcriptome during full-scale (3275 hL) lager wort fermentation were measured with the aid of oligonucleotide-based DNA arrays and were compared to changes in the fermentable carbohydrate and amino acid composition of the wort. Of the 32 genes involved in transmembrane transport of amino acids, all showed statistically significant changes in expression, with maximal transcription typically coinciding with amino acid limitation. Genes encoding the low affinity amino acid permeases displayed differential transcription profiles, suggesting a synchronized functionality, with at least one transporter operational at any given time. Genes involved in sugar transport similarly demonstrated a significant differential change in transcription. The *HXT* and *MAL/MPH* genes, which encode proteins involved in the transmembrane transport of sugars, displayed transcriptional profiles consistent with their susceptibility to carbon catabolite repression and the gene products' biochemical affinities for sugars. A notable exception was the *HXT4* gene, which had relatively high transcriptional activity under high sugar conditions, despite being a high affinity glucose transporter. The transcriptional changes observed are discussed in relation to their significance to brewery fermentation, yeast metabolism and flavor development.

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Investigation of a floatation process in the respect of oxygen consumption by yeast and ester control

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We use a flotation process with a dedicated tank for cold break removal. This method, however, involves tank cleaning after each brew and, consequently, results in considerable costs for rinse water, energy, and detergent among other things. Therefore, we considered the possibility of a brewing method that allows the omission of the floatation tank without compromising product quality. A test brew using a pilot plant revealed that cold break removal had little influence on any of the values in the analysis or on flavor quality, such as bitterness and astringency. On the other hand, in a full-scale brewery test brew without a flotation tank, a larger amount of acetate esters was produced than when the conventional brewing method was used. Following this result, an additional test was performed in which the wort aeration rate was increased to increase the amount of oxygen available for consumption by the yeast. As a result, the amount of product esters decreased, and the flavor quality remained equivalent to the quality obtained by the conventional method. When aerated wort is allowed to settle in a flotation tank, a sufficient amount of oxygen can be stably supplied to the yeast for each brew batch. On the other hand, in full-scale brewing where a fermentation tank is filled with several brew batches, when aerated wort is newly poured into the fermentation tank, the oxygen in the wort will also be consumed by the yeast in the pre-existing wort in the fermentation tank. Consequently, the amount of oxygen available for consumption by the yeast (especially that available for yeast added later) is expected to decrease and result in the increased production of acetate esters. We found the possibility that the flotation process could be in-

volved in the control of ester levels. If we manage to suppress the production of esters by increasing the amount of oxygen consumption by the yeast, we would be able to omit this process. This is one example that our pursuit of efficiency resulted in finding a clue for technology development.

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Dried yeast: Impact of dehydration and rehydration on brewing yeast cell organelle integrity

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As a consequence of drying, yeast cells are susceptible to damage, which primarily occurs due to water loss. Associated effects can include cell wall crenellation, cytoplasmic crowding, DNA super-coiling, membrane disruption, phase transitions and ultimately cell death. Although the dehydrated phenotype has been well characterized, the sequence of events that cause damage to the cell have not been effectively investigated. To address this we have studied the impact of dehydration and rehydration on three key attributes that are critical to brewing yeast quality and performance at the onset of fermentation: viability, genome stability and plasma membrane integrity and function. In the current study, the impact of dehydration on the stability of the brewing yeast genome (including both chromosomal and mitochondrial DNA) was established by analyzing restriction fragment length polymorphisms and chromosome length polymorphisms in dried and rehydrated populations, in addition to laboratory grown cells. Plasma membrane integrity and functionality (fluidity, H+ATPase activity and composition) were also investigated using fluorimetry and proton efflux evaluations.

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Functional analysis of mitochondria in fermentation: Role of mitochondrial DNA (mtDNA) copy number in resistance of brewing yeast to fermentation stresses

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Brewery fermentations and handling of yeast populations between successive fermentations exposes brewing yeast cells to a number of biological, chemical and physical stresses. It is generally accepted that repitching of yeast in subsequent fermentations leads to an increase in incidence of petite mutations, which result from the loss of mitochondrial DNA (mtDNA) integrity. Eventually this can lead to aberrant fermentation profiles and impaired product quality. Ale and lager yeasts exhibit different susceptibilities to elicit stress and repair responses to the conditions which favor petite formation. Since all mtDNA must be damaged for a petite mutation to be formed, susceptibility of a given strain to forming petite mutations may also be a function of the mtDNA copy number (typically 20–50 in *Saccharomyces* species). We have explored the effect of serial repitching of warm and cold cropped yeast from production scale cylindroconical vessels on mtDNA copy number using real time PCR and % petite mutations. Samples were collected from different portions of the cone, and mtDNA copy number was shown to vary. Restriction fragment length polymorphism (RFLP) assessment of petite mutations isolated from different crop generations showed variability, demonstrating the instability of the yeast mtDNA when exposed to stress in the cone. The role of oxidative, ethanol and acetaldehyde stresses in mtDNA copy number and mtDNA integrity has been assessed and hence the propensity of yeast to form petite mutations.

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The development of a simultaneous measurement of yeast viability and vitality by flow cytometry

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Yeasts with high vitality are very important for brewing high-quality beer. Accordingly, techniques for yeast vitality measuring are considered to be basic to the understanding of the yeast condition, which can lead to the production of high-quality beer. In the many methods that have been developed until now, the intracellular pH (ICP) method, which achieves the highest sensitivity by using the principle of H^+ extrusion activity, has been used for handling of high activity yeasts in our breweries. However, the ICP method targets only "vitality" and not "viability". Consequently, low-viability yeasts with 99% dead cells and 1% high-vitality yeasts might be determined to be active yeasts. In this study, we combined two different concepts of vitality and viability and developed a new technique that measures them simultaneously, which could solve these problems. This technique uses a flow cytometer with the ICP method for measuring vitality and a method using TO-PRO 3 (TP3), which enables the determination of viability by measuring the permeability of the plasma membrane of the yeast. In the ICP method, a pH-sensitive fluorescence reagent "5(6)-carboxyfluorescenceindiacetate (CFDA)" with an excitation wavelength of 488 nm is used, and for the viability measurement TP3 with an excitation wavelength of 633 nm is used. TP3 can stain the nucleic DNA by using its permeability through the plasma membrane of the yeast, which enables very sensitive viability measurement without interference with fluorescence of CFDA. We could accurately analyze both vitality and viability simultaneously by flow cytometric measurement after the treatment of yeast suspensions with CFDA and TP3 in citrate-phosphate buffer (pH 3). In contrast to the existing methods that cannot provide vitality and viability measurements of the same yeasts group, this method offers an accurate simultaneous measurement of "vitality" and "viability" which combines the two different concepts of yeast's physiological state.

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Improving beer flavor and fermentative capacity with selected beer yeast produced on maltose medium

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Maltose, maltotriose and glucose are the most abundant fermentable sugars in wort; in the case of incomplete fermentation, maltotriose can cause a range of qualitative problems in beer and ethanol loss. Furthermore, yeast which comes pre-grown on glucose biomass cannot fully adapt itself during beer fermentation. The development and production of selected beer yeast for fast and complete metabolization of these three main fermentable sugars in wort has been considered. The performance of fermentation is followed through the optimization of the culture medium, reproducing accurately the wort composition by monitoring yeast growth, ethanol synthesis, original gravity and attenuation, and sugars consumption during the fermentative process. Beer flavor was evaluated through the content of higher alcohols, volatile esters and aroma compounds. This study demonstrates that the selected beer yeast *Fermaltose* obtained from maltose biomass confers a more stable metabolism, a faster fermentation even in the case of nitrogen, lipids or vitamin deficiency, an improved maltose and maltotriose conversion, resistance to ethanol and temperature impact. The equilibrium and reproducibility of the aromatic profiles have also been analyzed, in comparison with traditional yeasts after successive inoculation: mutation, membrane permeability, study of the permease.

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Nitrogen source starvation induces expression of Lg-*FLO1* and flocculation of bottom-fermenting yeast

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In brewing, bottom-fermenting yeast flocculates after fermentation finishes. It is advantageous to brewers because sediment yeast is required for subsequent fermentation. This flocculation property seems to be inducible. However, it has not been clear what kinds of factors are involved in induction of flocculation. We investigated whether bottom-fermenting yeast flocculated under different starvation conditions. Only in the case of nitrogen source starvation did bottom-fermenting yeast flocculate. Not only in

the case of nitrogen source starvation, but also in the case of a non-preferred nitrogen source such as proline did bottom-fermenting yeast flocculate. From these results, it was considered that flocculation of bottom-fermenting yeast was controlled by a mechanism similar to NCR (nitrogen catabolite repression). The expression of Lg-*FLO1*, which caused flocculation of bottom-fermenting yeast, was controlled similarly. From these results, it was supposed that nitrogen source starvation and non-preferred nitrogen source induced Lg-*FLO1* expression and caused the flocculation of bottom-fermenting yeast.

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Detection of yeast in brewery rinse water

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Typically, vessels utilized within the brewing industry are sterilized or sanitized after use to prevent contamination from unwanted particulate matter, chemicals or microbes. The type and composition of cleaning agents can vary significantly between breweries but typically include hot caustic soda, steam, chlorine based sanitizers or acid agents such as peracetic acid. While the efficiency of such cleaning agents is typically good, it is common practice to perform tests to ensure that vessels are microbiologically clean. Analysis of water used to rinse vessels after sanitation can be performed to indicate whether any microbial contamination remains in the vessel and to ensure that hygiene standards are met. Although traditional methods based on cultivation are still employed in many breweries, these techniques are typically slow and only provide a result after a delay of several days or weeks. Recently there has been a growing trend toward the implementation of quick and reliable PCR-based methods for the detection/identification of bacteria or wild yeast contaminants in beer or process samples. However, in many instances pre-enrichment for 16–72 h is required prior to analysis and the level of differentiation provided is excessive for basic hygiene assessment. Here we describe a simple Q-PCR based method for the detection of yeast in rinse water samples as a means of assessing vessel hygiene. The method described includes the use of PCR primers designed to detect and identify *Saccharomyces cerevisiae* yeast. In addition, we demonstrate the application of a novel hollow fiber filtration module (Elutrasep™) which allows the accurate recovery of cells from a large sample volume. As such, pre-enrichment of process samples is not necessary, leading to a significantly faster response time. Here we demonstrate that the PCR protocol described may be used to routinely detect yeast present in rinse water samples. Consequently, a rapid assessment of microbial loading can be performed, aiding the implementation of effective HACCP monitoring and allowing proactive decisions to be made regarding vessel hygiene.