World Brewing Congress 2000

Poster Abstracts

P-1 Barley and malt pentosans: Structure and functionalities in the brewing industry

C. Leclercq, G. Dervilly, L. Saulnier, N. Dallies, D. Zimmermann and C. Roué

Pentosans are present in barley endosperm cell walls. These components show a high capacity for water retention, and could be involved in filtration problems. Barley and malt soluble pentosans were purified and fractionated by size exclusion chromatography. Our results showed that small fractions of high molecular weight were present in barley and malt, and that a solubilisation of these polysaccharides occurred during malting. The structure of these pentosans was analyzed by NMR. We also studied their functionality in wort by measuring the filtration capacity in Tepral wort. High molecular weight pentosans showed an elevated viscosity and affected wort filtration.

P-2 A rapid and simple extraction method for Fusarium DNA from infected wheat and barley malt

Sven Mulfinger, Ludwig Niessen and Rudi F. Vogel

Malt infected with toxigenic fungi, like *Fusarium graminearum* or *F. culmorum*, may render the quality of beer by the presence of mycotoxins or gushing. It is necessary to develop rapid screening techniques which can identify the relevant species within hours in order to reject contaminated malt. A new screening method was developed using a simple protocol for genomic DNA extraction, followed by species-species or group-specific identification using PCR. Toxigenic Fusarium species were identified on naturally infected wheat malt and barley malt samples. Signals for the PCR were well correlated to DON content in our experiments.

P-3 Automated control of barley respiration during germination

Leen Van Campenhout, Dirk Iserentant and Hubert Verachtert

Measurement of the respiration might allow a more reproducible control of the germination progress during malting than off line analysis of the grain. Therefore, barley was germinated in reactors in which the oxygen concentration of the inlet air could be controlled based on the difference between the actual outlet CO_2 concentration and a CO_2 profile to be obtained. The control strategy allowed to track CO_2 reference profiles, significantly different from the normal profile obtained during germination with a constant inlet air flow at 21% oxygen concentration. The effect of the control strategy on the natural microflora of barley was investigated.

P-4 A study of thermal gradient development in yeast crops

Gearoid Cahill, Padraig K. Walsh and Dan Donnelly

Ineffective temperature control of yeast crops reduces pitching yeast quality. Using a specially designed cooling rig, yeast crop temperatures were recorded over a range of distances from a cooling jacket (0.1–1.2 m). Thermal gradients of 3.5 °C were recorded within 5 hours of unmixed storage and these increased to a maximum of 11 °C. These gradients increased with increasing yeast metabolic activity. An illusion of good temperature control occurs in yeast crops with acceptable temperatures close to the cooling jacket and elevated temperatures towards the centre of the vessel. These findings can be used to optimize brewery cropping regimes.

P-5 Vicinal diketone reduction as a measure of yeast vitality

C.A. Boulton, W.G. Box, D.E. Quain and S.W. Molzahn

The majority of yeast vitality tests are calibrated using wort attenuation rate, or a related parameter, as the measure of fermentation performance. The rate of primary fermentation is clearly of importance, however, for many beer qualities, achievement of an appropriate VDK concentration controls vessel residence time. Furthermore, it can be shown that VDK accumulation and dissimilation is independent of the rate and extent of primary fermentation. Here is described a rapid test, which can be applied to pitching yeast and which measures the ability to reduce diacetyl. The effect of yeast physiological condition on diacetyl reduction is discussed.

P-6 Glycogen released by the yeast as a cause of unfilterable haze in the beer

Ph. Malcorps, P. Haselaars, S. Dupire and E. van den Eynde

Some plants were regularly faced with a persistent (29 °C) haze in the freshly filtered beer with some peak values up to 1.2 EBC. No clear relationship was found with bad filtering or brewing practices, changes in raw materials, or abnormal fermentation profiles. Palliative treatments like enzymes or fining agents were unsuccessful. The haze was identified as glycogen spontaneously released by the yeast during the fermentation. The phenomenon was strongly dependent on the yeast strain, its physiological status, the gravity of the wort, and the fermentation temperature. Glycogen particles could be present in every beer but at different levels. Their impact on the filterability and the clarity of the beer is discussed.

P-7 Oxidative destruction analysis of beer and intermediates

J. Savel and Dana Zdvihalová

Oxidative destruction analysis (ODA) of beer and intermediates is based on the typical beer properties investigation after oxidation with various oxidizing agents, mostly peroxodisulfates. Colour changes, haze development and off-flavour formation can be recognized very quickly during oxidation. The degradation of beer compounds such as amino acids, higher alcohols and other compounds can be proved. Oxygen free radicals and radical reactions are supposed to take part in ODA reactions. Chemiluminescence occurred and various products including volatile aldehydes were determined in beer and model solutions after oxidation. There is a possibility of quick beer properties stability testing and understanding beer ageing.

P-8 Development of easy-to-recycle colored glass bottles

Akira Shirakura

Conventional colored glass bottles are colored by the transition metal ions and it is difficult to obtain the colorless glass after re-melting due to the remaining of the ion. This study aims to establish the production technology of recyclable colored glass bottles which turn into colorless glass during re-melting process. This technology involves the coating of several micrometer-thick organic-inorganic hybrid material on the colorless glass bottles by use of the sol-gel technology. As the colorant, the film contains the organic pigment which burns and disappears easily on re-melting.

P-9 For Continuing Progress After the Anti-oxidative Beer Production System

M.Takashio and K. Shinotsuka

Sapporo has implemented the Anti-Oxidative Beer Production System and has been producing beers stable against staling. The compiled data so far was investigated further to find the next approach for improvement. The results were: 1) The resistant potential of beer against staling was improved by anti-oxidative production system. 2) Anaerobic mashing resulted in the difference of most indexes obtained from every following step of production, e.g. pH, CL, polyphenols, TN, amino acids, carbonyl compounds, and others with various magnitudes, and the correlation among the indexes was investigated. The reducing activities, which reflect various reactions, were practical as process control indexes. 3) As for the beer, besides the reducing indexes, 5-HMF, furfural and some unidentified compounds showed higher correlation with staling rate of stored beer, and the determination of these compounds brought significant information, for example, yeast performance, as well. 4) The phenomenon of temperature hysterisis of beer staling was scrutinized, and Thermal Control Transportation System was devised and introduced on the Japanese market effectively. Next steps of research, including the specification of raw materials and knowledge management system, are part of the on-going efforts at Sapporo Breweries Ltd.

P-10 Kettle Boil and 170-degree Control

Lee Hodge-Muse

Kettle Boil /170-Degree Water Control Project As energy manager at the Eden plant the efficient operation of the utility boiler is very important. One of the first issues brought to our attention by utilities staff was the effect of the constant and often dramatic swings in steam demand from brewing. These swings were coming from two sources, kettle over-boils and the 170-degree water make-up systems. These swings in the earlier days of operations often caused the boiler to be pulled off line. As the utilities staff grew in experience these occurrence decreased in number, yet the root cause remained. Utilities had learned to operate on the edge, out of necessity. It was their job to supply the brewery with steam. An inefficient boiler operation was the price of business. Brewing on the other hand had quality concerns. The boil set point is SOP, any deviation from that is unacceptable. Brewers are taught to demand a rolling vigorous boil. If the kettle was not foaming out of the door you were not boiling correctly. The only safeguard in this process are the over-boil probes. The operation of these probes is simple. If the foam hits the probe the steam to coils and steam to percolators slam shut. The steam will not come back on until the probe is cleared. It was not uncommon for the over-boil probe to be hit 30 times in the filling and boil process. Now imagine four kettles in various stages of filling, knocking out and boiling. Couple this with the needs of the mash-mixer. Then add the normal pasteurizer demands or grains drying pull. Boiler outages became common place even with a seasoned staff. The swings caused by the slamming of the steam valves due to over-boils or the sudden demand for 170 degree water could cause a swing in demand from as much as 25,000 to 60,000 lbs. of steam momentarily. This is enormous. The problems this caused the boiler were: 1.) Boiler draft issues. 2.) Boiler trips on low water. 3.) Scanning problems, black spots in the boil. 4.) Boiler pressure drops. 5.) Inefficient coal and or oil usage. Outages were more common when the seasons changed and we picked up the plant-heating load as well. The technology used in Eden to control the boil process was state of the art 20 years ago. As we researched ways of maximizing boiler efficiency we looked again at the brewing load. Technology had improved. There was a software company selling a program to breweries to control their kettles. After reading their literature we realized we could do a better program in house with our people. The next step was to develop a ROI. We looked at the amount of #2 fuel oil used to start a boiler that had tripped and the poor boiler efficiencies due to huge load swings and settled at a reasonable percent improvement in operations. With those numbers in hand a project was written and approved. The control of the kettle boil would require a high tech foam probe. We settled on the Drexelbrook Capacitance Probe. The software is a Sable program unique to the D3. (It is a sequence and batch language.) The kettles were already equipped with flow indicator on the steam to percolators. The steam to the coils was another issue. We settled on a new flow indicator by BEI. It is a new flow meter using microchip technology. We contracted with CA Jones & Son to write boil logic based on direction from us. The logic will be explained in detail in the final paper. The entire project was done for less than $ 60,000 and the results are extremely positive to date. The 6% evaporation issue is resolved only minor adjustments and tracking are required

P-11 Production of non-alcoholic beverages in a brewery based on cereals with bacteria

F.M. Briem

The paper/poster describes the idea to produce a probiotic alcohol free, fermented beverage in a brewery or a brewery-like factory. Fermentation organisms are two different bacteria isolated from

Kobucha which are used as pure fermentation cultures under defined aerobic and anaerobic conditions. These bacteria produce different organic acids and other fermentation by-products depending on fermentation parameters (temperature, oxygen saturation, substrate composition). As a starch source barley and wheat malt is used which is degraded during a conventional mashing programme to produce fermentable sugars for the bacteria. The resulting beverage is slightly sour, not too sweet and very refreshing. This beverage should be a good alternative for health conscious people to other soft drinks with the idea to consume a beverage with a high nutritional value with a low sugar concentration and high vitamin and mineral concentration. In September 99 we launched this product on the German market and the first results show us that there is a great acceptance by the consumers. The beverage is produced in a 20 hl brew house which gives us a total capacity of 30,000 hl per year. This product could help to bring a new aspect into the discussion of a breweries image.

P-12 Achievement of CFC-free operations at Asahi's Nagoya brewery

Y. Sasaki

HFCs had been a potential alternative to CFCs and HCFCs for prevention of ozone depletion, but was determined in December 1997 to be a greenhouse gas at the CPO3 held in Kyoto, Japan. In the wake of this decision, Asahi has achieved CFC-free operations at its Nagoya brewery by eliminating use of all HCFCs and HFCs and adopting natural refrigerants, such as ammonia, propane and water, in order to contribute to prevention of global warming and ozone depletion. The brewery also achieved a complete overhaul of its systems for energy use to enhance its energy and cost efficiency. The CFC-free operations were achieved at all equipment that used HCFCs and HFCs, including air conditioning, analytical devices, refrigerators, vending machines, and all brewing facilities. Some of the technologies newly introduced are as follows: (1) ammonia-absorption refrigeration units, (2) ammonia-screw refrigerators, (3) lithium bromide-absorption refrigeration units, (4) propane-based air-conditioning, (5) refrigerators using isobutane as a refrigerant, and (6) electronic coolers. We have carried out the following for improvement of energy and cost efficiencies: (1) cascaded heat transfer using gas-turbine cogeneration system, ammonia-absorption refrigeration units, and steam-driven back-pressure turbine refrigeration units, and (2) complete heat circulation using collection system for exhaust heat. The achievements from the above efforts are: (1) prevention of ozone depletion by the outright elimination of CFC refrigerants (HCFCs and HFCs) and (2) reduction of greenhouse gas emissions by enhanced energy efficiency and success in lowering costs.

P-13 The reduction of total Energy loss in Suntory Kyoto brewery

Yuji Doi, Hirofumi Hashimoto, Sadao Yamamichi, Minoru Yoshida, Masaaki Fujiwara, Tetsuo Morita und Yoshihiko Kakimi

The increasing demand for the energy saving has recently been emphasized in brewing industry from the viewpoint of not only cost reduction but also environmental protection. We have been making progress in energy saving at every section in brewery, energy supply section, energy using sections, and engineering section. We defined that the energy loss consisted of four parts in our brewery. Namely, (1) Power plant loss, (2) Supply loss, (3) Demand loss, and (4) Exergy loss. We have managed each energy loss at every section, and have made improvement programs to optimize the energy supply and usage in whole brewery. According to this strategy we have adopted absorption refrigerator, anaerobic wastewater treatment plant, co-generation system, and so on. We could succeed in remarkable result through these programs. Here we will introduce an example of these programs, in which we could reduce the Power plant loss and Exergy loss. We installed a high performance co-generation system, which had steam accumulator and waste heat boiler with duct burner (gas turbine exhaust supplementary firing burner) developed for this program. The main fuel of this burner is the bio-gas generated at wastewater treatment plant (anaerobic process). By means of this new combined system of co-generation and anaerobic wastewater treatment process, we could greatly reduce carbon dioxide gas emission and energy cost.

P-14 Beer and folates

C. Walker and E.D. Baxter

Currently a major health concern is that we humans do not get enough folates in our diet. Folates, which are part of the B group of vitamins, have recently been linked with the prevention of cardiovascular disease and several types of cancer. Different forms of folate occur naturally in foods some of which are thought to be more active physiologically than others. Beer, unlike wine or spirits, is a good source of folates. It originates from the malt and so the amount and type of malt are key issues in determining the final folate content of beer. We have optimised folate analysis in such a way that it can be applied to beers and further, we can distinguish between different forms of the vitamin. It appears that not all beer brands are created equal! This paper will examine differences in folate content between beers and will discuss the extent to which these may be related to differences in raw materials.

P-15 Alcoholic fermentation of rice with supplementation of inactive dry brewer's yeast

W.J. Lee

Six milled rice samples (5 Japonica, 1 Indica) were used to evaluate the quality of brewing raw materials. Indica sample had higher amylose and protein content and lower starch content than Japonica samples. A grist of milled Japonica rice was liquefied with the heat stable alpha-amylase at 65, 80 and 95 °C. The degree of starch hydrolysis was quantitatively determined by the iodine affinity method. The highest degree of starch hydrolysis was obtained at 80 °C. After liquefaction at 80 °C for 1 hr, mashes were saccharified with amyloglucosidase at 60 °C for 1 hr. Mashes contained 16.6 g of dissolved solids per 100 ml and 10 mg/L of free amino nitrogen and mashes were fermented with active brewer's yeast. Rice mashes were supplemented with inactive dry brewer's yeast at 100 mg free amino nitrogen/L and compared with unsupplemented mashes in terms of fermentation time and ethanol yield. Rice mash prepared by adding 1 kg of grains to 4 liter of water produced over 8.7% v/v ethanol within 4 days of fermentation at 25 °C with an inactive dry brewer's yeast supplementation.

P-16 Control of ester synthesis during brewery fermentation: myth or reality?

J.-P. Dufour and P. Malcorps

The synthesis of esters by yeast is of major industrial interest because it determines the fruity aroma of beer. The need to understand and control ester synthesis is driven by problems encountered in brewing procedures such as high-gravity brewing (which produced disproportionate amounts of ethyl acetate and isoamyl acetate), large scale CCT (reduction of ester levels) or the production of reduced alcohol beers (lack of flavour compounds). In 1962, Nordstrom demonstrated that esters are formed via an intracellular process catalysed by an "ester synthase". Since then, our practical understanding of yeast ester synthesis has advanced considerably. Technological parameters that affect the production of esters can be divided in 3 categories: those related to yeast characteristics, to wort composition and to fermentation conditions. Recently, scientists have taken advantage of the completed *S. cerevisiae* genome sequence database and the powerful tools of molecular biology to determine the physiological role of ester synthesis. Recent rapid progress has provided insights not only into the regulation of cellular ester synthesis, but also into some general mechanisms of gene regulation. Evidence from gene disruption and expression studies suggest that the different ester synthases may be involved in very different functions, including cellular fatty acid homeostasis and detoxification mechanisms which protect the cell against certain toxic compounds. An understanding of the molecular basis of ester synthesis should allow for better control of ester production in beer.

P-17 A new method to detect the reason of problems in filterability of wort and beer

S. Kreisz and W. Back

Poor filterability of beer can cause production failures and economic disadvantages. This paper will introduce a new method to control the development of filterability of wort and beer during the whole production process and a way to detect the origin of problems in filterability. The method is based on two new analysis. On the one hand a method to forecast the filterability of beer from wort published at the EBC 1999 in Cannes. On the other hand a possibility to identify the polysaccharides which causes filterability problems by releasing and identifying them from diatomic earth after filtration The filterability of wort and beer is measured by a pilot scale filtration published by Raible in 1990. The wort is treated with Ethanol and citric acid followed by a storage at 32°F. The results show that this treatment simulates agglomeration and precipitation procedures which normally take place during fermentation and storage. If poor malt quality or wrong processing in the brewhouse causes problems in filterability it can already be shown by a poor filterability of the treated wort. If the wort or the beer show insufficient filterability the filtercake will be stored. Afterward the polysaccharides which are the main reason of problems are separated from the diatomic earth. Polysaccharides can occur from three sources: malt, yeast and bacterial contamination. Each polysaccharide owns a different structure and consists of different saccharins. By hydrolysing the polysaccharides and detecting the saccharins with HPLC it is possible to associate the spectrum of saccharins to the source of the Polysaccharide.

P-18 New filtration technique for the beer recovery from spent yeast

Jan Schneider and Horst Weisser

A new Technique of Microfiltration uses the oscillation movement of membrane discs to effect a shearing rate on the membranes surface. In contrast to the conventional cross flow filtration the energy for the relative movement is not transferred into the unfilterd medium but directly to the membrane. While the yield is comparable to conventional techniques some important benefits could be observed: In consequence of the technical principle the sensible yeast can be kept at low temperature. The analytical results indicates that the permeate is comparable to normal, filtered beer. Thus the recovered beer could be added even after the beer clarification. Furthermore the power consumption is low which is also due to the technical principle which utilize the coupled torsional vibration for providing the oscillation of the membranes. The filtration behavior is very different to conventional cross flow filtration since no membrane fouling can be observed up to a certain solid concentration. By topping this concentration the filtration show a behavior comparable to the membrane fouling. The presented technical and analytical results base on large scale trials in breweries. Summarizing this new technique indicates new interesting aspects for an economical recovery of spent yeast and for other brewery application which is also part of this research project.

P-19 Application of filterability-predicting apparatus for prefiltered beer to the actual production sites

Hiromichi Aoto, Kinya Kurihara, Kazuhiko Shimada and Fumihiko Yokoyama

At Kirin we produce a number of different brands of beer to satisfy the varying requirements of our customers. Each brand of beer possesses different filterability, and sometimes batch to batch variation in filterability is also observed within a brand when different lots of malt have been used. If we were able to predict the beer filterability prior to the filtration process, several problems that are encountered during filtration could be addressed. These include increase in filtration differential pressure, decrease in filtrate flow, decrease in clarity and insufficient product quantity, and could be solved by adjusting the filtration conditions, by for example altering the amount of filter aid added before the filtration. Previously, we have reported on the accurate prediction of filterability of prefiltered beer that could be carried out easily and rapidly without any error between different workers. We then developed a method to determine the causes of the poor filtration, for example, due to the presence of yeast in the pre-filtered beer, and these results were reported at ASBC in June 1999. We now report the development of a filterability-predicting apparatus based on our previously developed filterability-prediction method and its introduction and evaluation at nine of our production sites. Our results showed that the above apparatus was successful at all nine sites and also that the causes of poor filterability were different at each factory. These causes were separated into 3 groups: sites where yeast present in the pre-filtered beer was the only cause, sites where particles other than yeast was the cause, and sites having both yeast and other particles as the cause.

P-20 Selective protein adsorption from lager beers by silica gels

Kenneth Leiper, Ian McKeown, Graham Earl and Graham Stewart

Silica gel has been used for many years in brewing to delay the onset of colloidal instability, the main advantage being that it is able to remove haze causing proteins while not removing those which contribute to foam stability. This study shows that selective protein removal is affected by silica pore size and beer type. As haze-causing protein is known to contain high levels of proline, isolation and analysis of proteins recovered from spent silica gels can be used to assess their haze protein removal potential. Silicas with a range of pore sizes were compared in this way in order to find the optimum pore size for haze removal from four types of lager beer.

P-21 Barley as a source of 4-Vinylguaiacol off-flavour in lager beer

A. Doderer and E.R. Brouwer

4-Vinylguaiacol (4VG) is a phenolic compound that contributes to the unique and desirable flavour of wheat beers. However, in lager beers, too high a concentration of 4VG is considered an off-flavour that is perceived as "phenolic", "clove-like" or "smokey". 4VG is derived from ferulic acid, a constituent of the barley cell wall, by enzymatic or thermally induced decarboxylation. Top-fermenting yeasts, which are used for wheat beers, contain ferulic acid decarboxylase activity while bottom-fermenting lager yeasts lack this enzyme. As a consequence, the presence of the 4VG off-flavour in lager beers is largely due to microbial infections, including top-fermenting yeasts. Recently, a regional lager beer was found to contain consistently elevated levels of the 4VG off-flavour in the absence of microbial infections. We traced the source of the 4VG to barley varieties from specific growing regions. By using malt from these regions, we were able to consistently produce lager beers on both laboratory and production scale with elevated levels of 4VG. We are currently carrying out research to determine if the growing regions or the barley varieties are the main reason for the high levels of 4VG.

P-22 3-Methylthiopropionaldehyde as precursor of dimethyltrisulfide in aged beers

L. Gijs, J. Pellaud, C. Vermeulen, P. Perpète and S. Collin

Due to a very low threshold (0.1 ppb in beer), dimethyltrisulfide (DMTS) is a key flavor of aged beer. On the basis of literature, 3-methylthiopropionaldehyde was suspected as potential precursor of this onion-like off-flavor during beer ageing. First analyses of various commercial beers went in the same way: the higher the 3-methylthiopropionaldehyde in fresh beer, the higher the DMTS in aged beer. Spiking fresh beer, wort before boiling or wort before fermentation also led to higher levels of DMTS in aged beer. The influence of temperature and pH, during boiling and ageing, on the 3-methylthiopropionaldehyde degradation was also studied. All those analyses pointed the major influence of the choice of raw materials. A linear relationship was indeed found between malt color and 3-methylthiopropionaldehyde concentration in wort. But the presence of copper, iron, sulfur dioxide, ascorbic acid or riboflavin also could favor, or penalize, the production of DMTS during ageing.

P-23 Development of evaluation method for beer taste and texture by using a lipid-coated crystal quartz microbalance

H. Kaneda, K. Shinotsuka, T. Kobayakawa, S. Saito and Yoshio Okahata

The relationship between the adsorption of beer constituents on a lipid membrane and its sensory evaluation was studied to develop an objective evaluation method for beer taste and texture. A quartz-crystal microbalance (QCM) coated with dioctadecyldimethyl-ammonium poly (styrenesulfonate) was connected to an oscillator designated to drive the quartz at its resonance frequency in an aqueous solution. The lipid-coated QCM was soaked in distilled water or buffer solution for 30 s to check the frequency stability, and then the degassed beer was injected with stirring. Frequency changes in the QCM were followed with time due to the adsorption of the beer components onto the lipid matrix on the QCM. The adsorption values of several types of beers showed a significant correlation with their bitter intensity, bitter duration, body, or smoothness in a sensory evaluation. It appears that the sensory bitterness, body, and smoothness can be objectively evaluated by measuring the adsorption characteristics of beer on the lipid membrane which simply modifies the gustatory and texture reception systems in the tongue and throat.

P-24 Influence of branched-chain amino acid aminotransferase on the production of different volatile compounds derived from the Isoleucine-Leucine-Valine pathway

L. van Nederveld, N. Benvenisty and A. Debourg

The Isoleucine-Leucine-Valine (ILV) pathway has been shown to contribute to the formation of higher alcohols and vicinal diketones. The availability of *Saccharomyces cerevisiae* mutants in which the genes ECA39 and ECA40 coding for the 2 branched-chain amino acid (BCAA) aminotransferase have been deleted offers the possibility to further defining the role of these enzymes in the formation of higher alcohols. As propanol is synthetised from ketobutyrate, the first metabolic intermediate in the anabolic pathway of isoleucine, nor eca39 nor eca40 mutations have any effect on the production on this higher alcohol. On the other hand, it can be concluded that eca40 mutation has a drastic effect on the production of isobutanol. As ECA40 is coding for the cytosolic BCAA aminotransferase, this enzymatic activity could be responsible for the transamination of valine in the ketoacid precursor of isobutanol. To a certain extend, the same conclusion can be made for the production of active amyl alcohol and isoamylalcohol, although the results suggest that one other route could lead to the formation of these two higher alcohols. Indeed, these 2 higher alcohols are still present in fermentation medium of strains having both BCAA aminotransferase mutations and a mutation in the isoleucine-valine or leucine pathway. Moreover, the eca40 mutation leads to an unexpected decrease of the production of vicinal diketones.

P-25 A new stale flavor index to improve flavor stability

S. Araki, M. Takashio and K. Shinotuka

The behavior of hop bitter substances during storage was studied using HPLC. It was difficult to detect any change in the bitter substances, but a significant difference was observed in the decreased rates among the HPLC peaks during beer storage. We chose two peaks from the HPLC analysis. They included peak A

that decreased with beer storage time and peak B that remained unchanged. The A/B ratio based on their peak areas was almost similar in fresh beer regardless of the beer specification but decreased in aged beer, which differed with the beer. It was also confirmed that a good correlation between the A/B ratio and stale flavor intensity of the beer, such that the A/B ratio reflected the degree of oxidative deterioration of the beer. Thus, this A/B ratio successfully gave a quantitative score to the staling degree of the stored beer. It is significant that the A/B ratio of the force-aged beer was a good indicator to predict the stability of the beer versus staling and evaluate the measures to produce a stable beer against staling. The change in the A/B ratio by minimization of oxygen uptake during mashing and the structural information of peaks A and B will be disclosed.

P-26 Evaluation of alcohol beverage by odor sensor

S. Shindo, M. Kumagai, S. Watanabe and S. Takahashi

A beer and a Japanese sake were analyzed by the odor sensor. In this experiment, AromaScan MultiSamler SP was employed as the odor sensor. The condition of sample preparation using alcohol beverage affected to the data reproducibility. The sample preparation was done under the following conditions. A portion of each sample,1.0 ml was placed into a vial contained 6 g of sodium chloride and the vials were capped. When the flavor volatile such as esters in the 15% ethanol solution were analyzed using odor sensor under the optimum conditions, isoamylacetate, ethyl capryrate, and isoamyl alcohol were discriminated as different samples. When the quality of 35 samples of Japanese sake were analyzed by odor sensor and compared to the results of the expert panels, the coefficient of correlation between the both results was 0.75. Above results suggested that the classification of the quality of Japanese sake is possible using odor sensor in replacement of human panel. On the other hand, discrimination between the fresh beer and stale beer was very good when beer was analyzed by odor sensor under the optimum conditions. Furthermore, the smell of beer stored under 20 degree centigrade for 10 days and that of beer stored under higher temperature (up to 37 degree centigrade) for 10 days were another oxidized smell by odor sensor. This results agreed with the results of the panel analyzing.

P-27 Biogeneration of flavours: Performances of *Candida methanolovescens* strains in non- and low-alcoholic beer

K. van den Bremt, I. van der Plancken, F. Delvaux, H. Verachtert und G. Derdelinckx

The beta(B)-glucosidase activity of Torulopsis sp. and *Hansenula sp*. has been demonstrated to be suitable in fields like winery and fruit juice production to improve the flavour bouquet of the beverages by liberating aglycons (like terpenes and alcohols). Further, methylotrophic yeasts were shown to be useful by a more complicated pathway involving oxidation of alcohols into the corresponding aldehydes that are characterised by a much lower threshold. By growing *C. methanolovescens* mutants – selected after a natural treatment – on a substrate like glucose, it was demonstrated that alcohol oxidase activity is present while its synthesis is totally repressed in the wild type strain. The presented results show the possibility of using the selected *C. methanolovescens* strains, which posses both B-glucosidase and alcohol oxidase activity, as pure culture and as mixed culture with *Saccharomyces* strains in the production of non- and low-alcoholic beer.The influence of the use of these micro-organisms on the sensory characteristics are described by GC-analysis of their higher alcohols, aldehydes, esters and organic acids, and by tasting. The foam stability is unaffected and shelf life is improved. The feasibility at lab scale of this approach is demonstrated and gives clear indication for further scaling up.

P-28 New measurement method for hop aroma component in beer and wort by SPME-GC-MS

T. Kurihara, S. Sakashita, T. Oshima, M. Kobayashi and K. Shinotsuka

It has been considered that characteristic hoppy aroma of beer is due mainly to the mono- and sesquiterpenes such as myrcene, linalool, humulene which are originated from hop oil. In flavor analysis of beer, according to the large amount of fermentation product, it was difficult to detect the hoppy aroma components selectively in conventional way. Thereupon, we developed the new method for the selectively detection of volatile components from hop by combining with the solid phase microextraction (SPME) and GC-MS technique. The volatile aroma components were extracted with the SPME and injected to GC-MS. Hoppy aroma components were selectively detected by selected ion monitoring with characteristic fragments such as m/z67, m/z93, m/z109, m/z138, m/z204 and m/z220. The peak intensity of selected ion's chromatogram was correlated with the evaporation rate at wort boiling and the sensory evaluation about hoppy aroma in beer.

P-29 Analysis of supercritical CO_2 extracts of hops — Variety related fifferences between ASBC spectro and HPLC values for alpha and beta acids

J.E. Carr und L.H. Parker

Hops extracts are most commonly produced and sold on the basis of their measured content of alpha acids. Yet, the two most common methods of analysis in the United States, UV Spectrophotometric (ASBC Spectro) and High Performance Liquid Chromatography (HPLC), frequently give substantially different values, the Spectro result normally being the higher of the two. To determine whether such differences are predictable, and perhaps related to the hop variety processed, two year's worth of analytical data was collated, comparing UV Spectro and HPLC (ICE-2 standard) values for alpha and beta acids for four high alpha bittering hop and two low alpha aroma hops. As suspected, the difference between Spectro and HPLC values for alpha acids content was indeed found to be variety dependent. For example, Nugget extracts had an average difference of around 8%, although the coefficient of variation (CV) was 40%. More specifically, at least for the high alpha hops included in this study, the Spectro/HPLC ratio was determined to be inversely proportional to the cohumulone ratio. Curiously, one aroma variety (Mt. Hood) extract actually had HPLC alpha about 5% higher than the Spectro alpha (CV 40%), even though the raw hop Spectro alphas were higher than the HPLC values.

P-30 Investigations into the production of a xanthohuhumol-enriched hop product

M. Biendl, R. Eggers, N. Czerwonatis und W. Mitter

Current pharmacological studies show positive aspects for xanthohumol and related prenylflavonoids of hops, e.g. possible prevention of osteoporosis or arteriosclerosis as well as anti-cancerous activity. The xanthohumol content of hops is a varietal characteristic and has been measured at between 0.2% and 1.1%. Via the combination of primary hop extraction with ethanol, followed by fractionating of the pure resin extract with supercritical CO_2, a partial separation of alpha- and beta-acids from xanthohumol can be achieved, resulting in a hop product that is enriched in xanthohumol. The content is up to 10%, depending on the hop variety used. Pilot plant evaluations were carried out. The process was realized by the new technique of high pressure spray extraction. A design for a continuously working hop extraction plant using this technique is presented. Future demand for a xanthohumol-enriched hop product could arise to be used as a beneficial ingredient for foodstuffs or as a nutraceutical. Brewing trials with the xanthohumol-enriched hop product show possible applications in beer production.

P-31 Evaluation of Near Infrared Calibration Performance for hop analysis at harvest

Scott W. Garden, Tamara Pruneda and David W. Hysert

Near infrared (NIR) calibrations have been developed for the determination of alpha-acids, beta-acids, and hop storage index (HSI) in hops. Calibrations were developed using the ASBC ultraviolet spectrophotometric method as the reference laboratory method. The calibrations were implemented and their performances evaluated during the 1999 harvest season. The standard error of prediction (SEP), coefficient of determination (r^2), and bias (i.e. average difference between laboratory and NIR analyses) were the calibration statistics used to evaluated calibration performance. For hop samples with alpha-acids concentrations greater than 7%, the SEP values reported for alpha-acids, beta-acids, and HSI predictions were 0.45%, 0.27% and 0.012, respectively. For hop samples with alpha-acids concentrations less than 7%, the SEP values reported for alpha-acids, beta-acids, and HSI predictions were 0.23%, 0.18% and 0.020, respectively. Calibration performance was variable when individual hop varieties were examined. For alpha-acids predictions, calibration performances were strongest (SEPs less than 0.24%) when predicting alpha-acids concentration in low alpha-acids (aroma) varieties (e.g. Willamette and Mt. Hood). Higher SEP values for alpha-acids predictions were observed with high alpha (bitter) varieties such as Galena (SEP = 0.35%) and Nugget (SEP = 0.39%). The weakest alpha-acids predictions were reported for certain super high alpha varieties (e.g. Columbus/Tomahawk and experimental varieties) with SEP values in excess of 0.57%.

P-32 Genetic history of hops

Atsushi Murakami, Toshihiko Takeuchi and Motoo Ohkochi

When and how did hops, *Humulus lupulus L.*, appear on the earth? What degree of genetic diversity was caused by the differentiation within hops, which resulted in European, North American and Japanese wild hops? This degree of diversity is closely associated with feasibility of hop breeding programmes. To answer the above questions, we studied DNA differences among various hops including typical cultivated, wild and related species. From the nucleotide difference of chloroplast DNA (cpDNA), the divergent time between hops and related species, *H. japonicus* was estimated to be approximately four million years ago. The differentiation within the hops occurred very recently, because only a few differences were observed in non-coding regions of cpDNA and ribosomal DNA spacer region. This may indicate a limited availability of the genetic diversity within hops. However, results of this study also suggested the centre of origin of genus Humulus is East Asia, where there is the possibility of finding a wide range of genetic variation or unidentified related species. We believe that the search and collection of new gene resources in the above area will contribute to the expansion of genetic variation, which may become necessary in hop breeding in the coming century.

P-33 Assessment of beer oxidation using long-path color techniques

Richard Pflugfelder

Beer color measured at 430 nm in a 1 cm cuvette is the only direct absorbance technique applied to beer in many breweries. The use of 5 or 10 cm cylindrical cuvettes for pale beers greatly enhances sensitivity to small color changes and reduces variability and random errors. Increasing reddishness in pale beers, resulting from oxidation of polyphenols, can also be measured accurately over a long optical path. A ratio of absorbances in the amber and red ranges ("Color Ratio") has proven to be a sensitive index of beer oxidation. A characteristic range of Color Ratios, from fully reduced to force-oxidized, has been found for each beer studied. Decrease in Color Ratio from the maximum (reduced) value reflects the extent of cumulative beer oxidation in process and package. Since blending has little or no effect on Color Ratio values, this technique can potentially be used to track oxidation state changes from fermentation through the end of packaged beer shelf life. SRM beer color (430 nm) also becomes a very useful parameter when measured over a long optical path. SRM color of pale beers increases significantly with age. Oxidation accelerates that color change. Long-path SRM color can also be used to detect mixed lots of similar products. A detailed outline of the techniques and relevant data will be presented.

P-34 Quantitative analysis of beer aromatic alcohols using stable isotope dilution analysis

J.P. Dufour, M. Leus, G. Lissens, F. Delvaux and D. Larsen

The aromatic alcohols, phenylethanol, tyrosol and tryptophol, are some of the higher alcohols produced by yeast during fermentation. Production of these alcohols is increased at higher fermentation temperatures (accelerated lager fermentation, ale fermentation) or when using a low FAN level wort. While phenylethanol has a pleasant fragrant aroma, tyrosol (bitter, chemical) and tryptophol (solvent, almond) are considered to contribute negatively to the beer flavour. The phenylethanol level has also been shown to suppress dimethylsulfide perception. Review of the literature reveals a large discrepancy in the threshold values of tyrosol and tryptophol in beer. Part of the variation may be attributed to the lack of a reliable analytical method for these aromatic alcohols. Our aim was to develop a quantitative assay of

beer aromatic alcohols using gas chromatography in conjunction with stable isotope dilution analysis (SIDA). Quantification using SIDA is not subject to instrumental and/or sample manipulation variation (extraction, concentration). The major advantage of this method lies in the closely matched physico-chemical properties of the aromatic alcohols and their corresponding isotopically substituted analogs. A range of beer samples were spiked with a known amount of deuterated aromatic alcohols. These were then extracted with different organic solvents. The concentrated extracts were analysed on a DB-1701 column, and the beer aromatic alcohols were quantified by GC-MS using the corresponding deuterated aromatic alcohols as internal standards.

P-35 Development of a dye-binding assay for haze-active protein in beer

Jing-Iong Yang and Karl J. Siebert

The objective was to develop a dye binding assay specific for haze-active (HA) protein in beer that would not suffer from the disadvantages of the turbidimetric assay (limited linear response range and the influence of endogenous polyphenols). A total of 15 dyes that resemble polyphenols were screened to see if any would produce a chromic shift upon binding to the HA protein gliadin. Gliadin is commercially available and very similar in amino acid composition to barley hordein, the source of the HA protein in beer. Four dyes were selected for further screening against a range of proteins. Bromopyrogallol red (BPR), was chosen for method development. In buffer, BPR produced an essentially linear response to gliadin. When BPR was applied to beer, however, its response was largely masked by interferences. A two stage pretreatment of beer samples, consisting of solid phase C18 adsorption followed by centrifugal concentration (10 kDa MWCO) before the BPR dye addition, removed the interference. The effects of treating unchillproofed beer with various levels of adsorbents (silica gel, bentonite and PVPP) and fining agents (gelatin and tannic acid) on foam-active and HA proteins (measured by both the turbidimetric and BPR methods) was studied. The results of the two HA protein methods were slightly different. The BPR method is quite advantageous in investigating the effects of stabilizing treatments, particularly those of fining agents.

P-36 Analysis of food-originating foreign materials in beer

Masato Kawasaki, Hitoshi Chiba, Shuso Sakuma and Motoo Ohkochi

Food is a major source of foreign materials in packaged beer. Since most foods consist of carbohydrate, protein or lipid, the presence of these materials can be detected by FTIR microscopy. However, in order to be able to respond to consumer complaints, it is also necessary to determine what kind of food the foreign material originated from. Therefore we have carried out an investigation to develop methods to enable us to identify these materials. When the FTIR spectrum indicated the presence of carbohydrate or lipid, the sugar or fatty acid composition was determined by chromatographic analysis. When the sample was found to contain proteins, SDS-PAGE analysis was carried out. Examination of the characteristic patterns of sugar, fatty acid or protein composition enabled us to identify the sources of foreign materials. FTIR microscopy in combination with these analytical techniques can be used to identify a wide variety of food-originating foreign materials.

P-37 Kinetics of light induced formation of 3-methylbutene thiol in beer

Michael J. McGArrity, David Maradyn, Robert Stewart, Don Thompson and Amanda Tinginys

When beer packaged in green glass is exposed to cool white fluorescent light, 3-methylbutene thiol forms readily. 3-MBT was monitored versus time. Its formation slowed as the reaction progressed until a maximum level, 3-MBT (f), was reached. The data give a linear first order plot (ln ([3-MBT(f)] – [3-MBT]) vs time) which is indicative of a first order rate law. The loss of riboflavin in beer was measured vs time and a first order kinetic plot was also obtained. (ln[Rb] vs time). When beers with ascending concentrations of isoacids were exposed, the resulting 3-MBT(f)s were found to be linearly dependent on isoacid concentration. During the kinetic runs, no appreciable loss of isoacids was observed. This is consistent with isoacids concentration being a pseudo kinetic term. Cool-white fluorescent light has line emission at 314 nm that is absorbed by green glass but is not by flint glass. When 3-MBT was monitored in beer exposed in flint glass, linear first order kinetics were not observed. 3-MBT formation continued after riboflavin has been largely depleted. These results suggest that, when beer is exposed to light with a short UV component, 3-MBT is formed by two mechanisms. One involves riboflavin photoxidation of isoacids to yield 3-methylbutenyl radical which abstracts sulfur to form 3-MBT. In the other mechanism, the intermediate radical is produced directly by fragmentation of photochemically excited isohumulones.

P-38 Detection of various barley, malt and beer proteins following SDS-Page utilizing three different stains

Sheryl Grider

Many different staining techniques have been used to detect barley, malt and beer proteins following SDS-PAGE. Of these various methods, there is no one single method of detecting all proteins in a sample. Coomassie Blue stain has an affinity for specific amino acid residues, which leaves other proteins undetected. Silver staining is a reliable technique for detecting most proteins, but leaves some glycoproteins undetected. To fully gain a fingerprint of barley, malt, or beer proteins, duplicate gels can be run: one stained with a polychromatic silver stain, and the other stained with a glycoprotein stain. Following the glycoprotein stain, the gel can be stained with Coomassie Blue to identify foam active proteins. The polychromatic silver stain stains different proteins different colors, hinting at the composition of the specific protein.

P-39 A statistical summary of malt parameter reproducibility in a Phoenix micromalting system

D.E.Langrell and M.J. Edney

Micromalting of barley lines entered in the Western Canadian Barley Cooperative Trials is performed in the Grain Research Laboratory for malt quality evaluation in support of the cultivar registration system. In order to do comparative quality analysis, consistency of malting conditions must be maintained within and among batch runs on the Phoenix micromalting system. To achieve a measure of consistency two check barley samples were malted with each batch, extending over a period of years. Malt analysis of these check samples run under the same conditions in the same

machine, has produced a clear statistical image of the system's reproducibility. Coefficients of variation were calculated for a number of malt analytical parameters, which showed variation in magnitude depending upon the sensitivity to changes in the system, and upon the precision of the analysis method itself. Ultimately, the inclusion of system check samples provides reassurance as to the validity of comparative quality data derived from malt produced by the system.

P-40 The effect of wort clarity on fermentation of all-malt wort

Stephen A. Martin and Graham G. Stewart

Effects of wort clarity on the fermentation of beer wort has been well studied and much knowledge regarding stability and flavour of the final product is now known. Enological studies have revealed that solid levels in wine must are an important factor in determining wine quality and this was attributed to differences in final higher alcohol levels. Initial studies have focused on differences in fermentation of normal and clarified worts. Results indicate that there are notable differences in the production of volatile, flavour-active compounds present at the end of fermentation. Subsequent studies have shown that clear worts maintain a higher concentration of CO_2 during fermentation, but diatomaceous earth alleviates this oversaturation to levels of CO_2 found in cloudy wort. There was, however, still a difference in the rate of sugar consumption. Also, cloudy worts contain a higher concentration of fatty-acids, although it was noted that clarified wort still contains some fatty-acids. Subsequently, different nutritional components found to be associated with wort solids have been added to clarified worts and their effect on fermentation assessed. Results show that nutritional components such as fatty-acids can exert an effect on fermentation, but alone cannot augment fermentation performance to the level of cloudy wort.

P-41 Surveillance of malting barley quality

A, Laitila and A.Haikara

Fungal flora characteristic to malting barley develops before harvest, under storage, and during the malting process. Fungi and their metabolites present in barley greatly influence malt and beer quality. We characterized the mycoflora on the Finnish malting barley. Fungal surveys were conducted during the years 1990 – 1999. Samples of dried barley (20 – 36 barley samples/year) were collected from local farmers during autumn. Over 5 seasons (1995 – 1999), 10 randomly selected samples were analysed for Fusarium-toxins. Samples of the 1998 and 1999 crops were analysed for ochratoxin A. The type and abundance of fungi was greatly dependent on the climatic factors such as total precipitation and temperature. Large variation in the infection level was detected in different years. *Fusarium* and *Alternaria* were the dominant fungal genera on Finnish malting barley. The amount of *Aspergillus* and *Penicillium* storage fungi was low due to the effective drying of barley by the farmers immediately after harvest. Ochratoxin A was not detected. Heavy rainfall during the summer 1998 promoted Fusarium growth. Although 75% of the samples exceeded 50% Fusarium-contamination level, the amount of mycotoxins was low. Zearalenon, nivalenol, 3-acetyldeoxynivalenol, fusarenon-X, diacetoxyscirpenol or T-2 were not detected, and only five samples contained low levels of deoxynivalenol (<30 ppb).

P-42 Optimization of the antioxidant activity of specialty malts for use in improving the flavor stability of lager beer

D. Bright, G. G. Stewart and H. Patino

Brewers have long been concerned with the flavor changes that beer undergoes between packaging and consumption. Increased globalization of markets and lighter beer styles are just two of the reasons many brewers are sharpening their focus on improving beer flavor stability. In this study the use of specialty malts to enhance the flavor stability of American-style lager beers was investigated. Malts were produced in a roasting drum under conditions designed to maximize the yield of antioxidant activity per unit malt color. Antioxidant activity was measured using the Total Antioxidant Activity (TAA) assay and a modification of the Indicator Time Test (ITT). Both assays were modified to separate as much as possible the strong antioxidants, such as the reductones from melanoidins. The malts were used at low levels in American-style lager beer and flavor stability was compared to controls. The specialty malt percentage was fixed below the flavor threshold against controls by adjusting the light malt roasting schedule. Incremental improvements in flavor stability were observed with the test beers.

P-43 Improvement of beta-amylase thermostability in barley seed

Naohiko Hirota, M. Kihara and K. Ito

In order to improve malting quality of barley, we developed a gene expression system in barley endosperm and generated transgenic barley producing thermostable beta-amylase. To construct gene expression vectors in barley endosperm, we isolated a D-hordein promoter. The promoter showed nearly the same expression level as the rice actin promoter in barley endosperm protoplasts. The thermostable beta-amylase expression vector driven by the D-hordein promoter, pDPTSAMY, was introduced into protoplasts derived from immature barley seeds. Of 16 plants from 6 independent transgenic lines, only one transgenic plant set T(1) seeds. Crude enzymes prepared from the T(1) seeds remained beta-amylase activity even after heat treatment at 62 °C for 30 min., where endogenous beta-amylase is completely inactivated. The activity of thermostable beta-amylase was accounted for 75.8 % of total beta-amylase activity. However, the total beta-amylase activities from the individual T(1) seeds were very similar to those from non-transgenic seeds. The mechanism responsible for the suppression of the endogenous beta-amylase activity is unclear, but we now speculate on the possible involvement of co-suppression in this phenomenon.

P-44 Activation of (1-4)-beta-xylan endohydrolase regulates the onset of arabinoxylan degradation during malting

M.P.M. Caspers, F. Lok, K.M.C. Sinjorgo, M.J. van Zeijl and V. Cameron-Mills

The importance of low wort viscosity for ensuring rapid and efficient wort filtration and recovery of wort extract is central to the economy of the brewery. The non-starch polysaccharides (1-3,1-4)-beta-glucan and arabinoxylan, present in barley grain cell walls, are solubilized during mashing leading to high viscosity. Although (1-3,1-4)-beta-glucan is largely digested in well-modified malt, the presence of significant amounts of residual arabin-

oxylan in beer indicates that degradation of this cell wall hemicellulose during malting is very limited. Our studies of the barley arabinoxylan degrading enzyme (1-4)-beta-xylan endohydrolase, focused on its synthesis, localization, activation and release from the aleurone, provide a new insight into the regulation of this enzyme and an explanation for the limited arabinoxylan degradation during germination. The major barley (1-4)-beta-xylan endohydrolase activity present in 11 day germinated barley, was found to be a 34 kDa protein. An isolated barley endoxylanase gene was found to encode a 61.5 kDa polypeptide, comprising an inner catalytic domain sequence homologous to the 34 kDa (1-4)-beta-xylan endohydrolase. The 61.5 kDa precursor is localized in the cytoplasm of aleurone cells from 3 days of germination. After a series of proteolytic cleavage events, the latent precursor is converted into an active 34 kDa (1-4)-beta-xylan endohydrolase, which is simultaneously released from the aleurone cell. Thus, delayed activation and release of (1-4)-beta-xylan endohydrolase within the germinating barley grain, can account for high residual arabinoxylan in barley malt.

P-45 Draft dispense systems and their relationship to beer quality

P. Benstein

Draft beer presents the brewer with a variety of difficult quality control issues. Because of the lack of complete brewery control, implementing change oftentimes is a daunting challenge. A microbiological sampling program was developed to study the draft systems of randomly selected accounts. Contamination changes were tracked during a 2-week cleaning cycle. A model, developed from the sampling results, was used to isolate some of the variables that degrade draft beers. Additionally taste panels were used to assess non-microbiological factors. Our results showed that freshly "cleaned" beer lines could still contain beer-spoiling organisms. Variables such as the standards for line cleaning activities, the age of the line, materials used for construction, and line installation methods played significant roles in determining the effectiveness of any draft beer quality control program. Other factors such as the inappropriate use of blended gas and poor installations caused negative flavor changes in the beers served from these systems. A Gold Standard Service Program for our draft beer was developed and implemented. By updating draft system cleaning technologies and by certifying organizations that deal with our draft beer, we have started to see significant quality improvements.

P-46 Specific and quantitative detection of *Pectinatus* by PCR using 16S-23S rDNA spacer region

Yasuo Motoyama, Takaomi Yasuhara, Tomoo Ogata, Toshifumi Yuuk and Noboru Kagami

Ever since *Pectinatus* were isolated in 1970s, the incidents caused by this organism have increased. This is likely related to the considerable decrease of oxygen content in beer brought about by advances in brewing technology, and to the increased production of lower alcohol, non-pasteurized beer. These industry trends will most likely continue to accelerate. Therefore, Pectinatus will be more harmful bacteria in 21st century. Several methods have been developed to detect *Pectinatus*, of which a polymerase chain reaction (PCR) is one of most useful, due to its sensibility and specificity. With PCR detection, the selection of primers is the most important factor in determining its specificity. We previously reported the sequences of the 16S-23S rDNA spacer regions of *Pectinatus* and found the homologies of these spacer regions were relatively low compared to that of the 16S rDNAs. In this study, we designated the primer sets from these regions, and confirmed these primer sets had a very high level of specificity. We also developed a quantitative competitive PCR method using internal control DNA, which was constructed by modifying the sizes of the wild-type amplified products and cloning them in plasmid vectors. The detection limits for two *Pectinatus spp.* were estimated to fall in the range from 1 to 20 cells/ml. This is the first reported application of the competitive PCR technique in the beverage/brewing industry. This method will be useful for quantitative detection of *Pectinatus* existed in beer, and for monitoring progression in the forcing tests.

P-47 Rapid detection of beer spoiling bacteria

Erik Bischoff and W. Back

Biological stability is still a problem for many breweries. Even the smallest spoor count of contamination suffices to ruin the image of a brewery. Hence, quick detection of beer spoiling bacteria is needed, especially in the spoor range. The aim of the system must be the detection of one viable bacterial cell per sample. The Polymerase-Chain-Reaction (PCR) is a method for detecting specific kinds of DNA in a short time. But for PCR a minimum cell count is required. So there must be a pre-enrichment before detection is possible. Due to variable lag-phases and the re-generation times of microorganisms, the pre-enrichment period must last at least 44 hours. The pre-enrichment media must provide good conditions for beer spoiling bacteria and it must inhibit harmless accompanying microorganisms. Problems may occur through *lactobacillus lindneri* due to a long lag-phase and by *pediococcus damnosus* due to a long re-generation time. The nucleic acid extraction's sensitivity for the PCR is crucial for the pre-enrichment time. This extraction has been optimized to two hours at a sensitivity of 1000 cells per sample for all kinds of bacteria. This verifies the existence of beer spoiling bacteria within 48 hours. In pilot projects in breweries it has become evident that it is possible to do a post-filtration verification within this time frame. For PCR unspecific primers can be used and the resulting signal may be analysed for an exact identification. This has the advantage that for the first analysis the expense in cost and time is much less than with a PCR with specific primers.

P-48 A preliminary investigation of optimal Microplate incubation temperature for Lactobacillus sp. using the Biolog Microbiological Identification System

Deanna W. Shoemaker, Lisa Beckler-Andersen and Paula L. Vann

Four Lactobacillus species (*L. brevis, L. plantarum, L. paracasei*, and *L. rhamnosus*) were identified using the MicroLog® Microbiological Identification System manufactured by Biolog, Inc. (Hayward, CA). MicroPlate incubation temperatures of 30 °C and 35 °C were compared for each species to determine whether there were any significant differences between identification results.

Statistical significance and variation of Similarity and Distance identification parameters were compared for each species at both incubation temperatures. Although *L. rhamnosus* and *L. paracasei* had better identification matches at the 35 °C incubation temperature, only *L. paracasei* had a significant difference between the temperatures for incubation. *L. plantarum*, having better identification results at the 30 °C incubation temperature, was the only other organism to show a statistically significant difference for species identification. Although all Lactobacillus organisms in this study could be identified at both the 30 °C and 35 °C incubation temperatures, identification results were better matched at a preferred incubation temperature for all but *L. brevis*.

P-49 Control of microbial contamination in continuous fermentation by immobilized yeast

I.A.I. Virkajärvi, T.T. Vauhkonen and E.L. Storgårds

To fully exploit the advantages of a continuous fermentation asepticity is a prerequisite. An early detection of microbial contaminations is advantageous whether the process is a batch or a continuous one. The high concentration of yeast in an immobilized bioreactor is a preventive factor, but it also complicates the detection of contaminations. We deliberately contaminated immobilized bioreactors with an enterobacterium, *Klebsiella terrigena* and monitored changes in yeast performance, viability, and in changes in chemical composition of the green beer. In particular, the formation of possible carcinogenic substances, biogenic amines, was monitored. *K. terrigena* caused elevated concentrations of dimethyl sulfide (DMS) in beer, but did not cause significant changes in analyzed higher alcohols or esters. On the other hand the ratio between diacetyl and pentanedione was increased. The contamination level in the reactor with porous glass beads was lower than either with beech or aspen chips. The yeast viability was lowered, but without observed changes in fermentative capacity. The increased concentration of biogenic amines was mainly due to changes in cadaverin concentration.

P-50 Granular activated carbon purification of brewing water supplies – Selection, usage, monitoring and replacement

Gil W. Sanchez

Water used for brewing must be fit for human consumption (potable) and satisfy Federal drinking water standards. It should also be aesthetic in appearance, taste and odor. Organic contamination arising from a multitude of causes can strongly impact the potable and aesthetic qualities of both surface water and ground water susceptible to surface contamination. Granular activated carbon (GAC) is currently the main treatment technique for removing organic contaminants, and in some cases, inorganic contaminants as well. However, GAC selection, usage, monitoring and replacement require careful consideration to be able to optimize its performance. Some of the key factors that effect GAC performance include the type and grade of carbon (coconut shell, bituminous coal, lignite, etc.), empty bed contact time and carbon filter operation. Because the carbon eventually becomes spent, procedures for monitoring its capacity and performance are important for ensuring acceptable water quality and determining carbon replacement rates without undue costs. This poster focuses on all of these aspects for granular activated carbon purification of brewing water supplies.

P-51 Minimizing oxygen content in bright beer tanks by use of acid cleaning

J. Dirksen and J. Duca

Oxygen levels in bright beer should be minimized to prevent oxidation flavors in beer. Oxygen content in the atmosphere of bright beer tanks is related to the type of cleaning program used to clean the tanks. The use of an acid cleaning and sanitizing program for bright beer tanks reduces the level of atmospheric oxygen compared to standard caustic cleaning. This study followed the oxygen levels in the atmosphere of bright beer tanks from the time it was emptied, then cleaned, sanitized, and refilled with beer. The study compared the impact of acid cleaning versus caustic cleaning in terms of dissolved oxygen content in the tank atmosphere and in the bright beer. A portable Orbisphere oxygen monitor was used to monitor oxygen levels in the atmosphere of bright beer tanks before and after cleaning and sanitizing. The Orbisphere monitor also measured oxygen content in the brewery's CO_2 supply. This paper describes a test methodology to connect the oxygen monitor to the bright beer tank, using a vacuum trap and vacuum pump to draw gas through the unit when the tank was not pressurized. The impact of headspace oxygen in the bright beer tank on dissolved oxygen content in beer is also discussed.

P-52 Effects of metal ions and gluconate on surface residues on returnable bottles

R.C. Cameron, A.T. Paulson, R.A. Speers, F. Hamdullahpur and W.F. Caley

When cleaning returnable beer bottles a white surface residue (film) is sometimes formed, and appears to be related to factors such as type of bottlewasher (soaker) and operational down time, as well as the type and quantity of dissolved and suspended solids and ion concentration in the caustic solution. The objectives of this study were to examine the chemical and physical conditions leading to the development of surface films, and their prevention. A model caustic soaker solution containing sodium hydroxide, sodium carbonate and sodium hexametaphosphate was formulated with either distilled (soft) or municipal (hard) water, and the effects of dissolved metal ions (aluminum, silicon and titanium) and amount of sequestrant (gluconate) on film formation were determined. Films were made on new glass bottles and glass microscope slides under simulated soaker time/temperature conditions, and examined for appearance by visual inspection, spectrophotometry and colorimetry. Metal ion levels were determined by atomic absorption spectrophotometry, and film microstructure was examined by scanning electron microscopy. The results showed that surface films could be successfully simulated in the laboratory. Film appearance and microstructure were significantly affected by solution composition. The effect of gluconate on film appearance and composition depended on gluconate concentration and the type of water and metal ions in the caustic solution.

P-53 Yeast cellular size and metabolism affecting flavor stability

C. Shimizu, T. Kimura, S. Araki, M. Takashio and K. Shinotsuka

The cellular size of yeast was found to be related to the fermentation performance by studying the set of yeast strains with different cellular sizes (average calculated from about 100 cells) derived from a certain clone of the bottom fermenting yeast. Besides the

fact that age and nutritional conditions affect the yeast cellular size, we have found that the difference in metabolic characteristics accompanied with the cellular size. We initially reported that the pH of finished beer correlated with the cellular size of the yeast for the same frequency of use. In this report, we show the other characteristics relating to cellular size, e.g., the produced acetates and organic acids and the uptake patterns of the amino acids, etc. The larger cellular yeast strain produced a beer with a higher pH and higher acetates in concentration compared with the beer produced by the yeast strain with a smaller cellular size. These phenomena observed during adjunct beer fermentation suggest that a common factor regulates the cellular size and yeast metabolism. From these results, the yeast cellular size is expected to be a type of marker for obtaining a strain that improves flavor consistency and flavor stability. Furthermore, we presumed that some spontaneous mutation resulting in the heterogeneity of the working yeast is caused by certain conditions, e.g. zinc concentration in the wort.

P-54 Serial repitching, yeast physiological state and brewing yeast fermentation performance

C. Jenkins, A. I. Kennedy, P. Thustron, J. A. Hodgson and K. A. Smart

Good yeast handling is an essential prerequisite for brewery fermentations. During the cycle of serial repitchings the yeast may be exposed to various physiological stresses, resulting in slurries of variable condition and subsequent inconsistent fermentation performance. The effect of limited and extended serial repitching on subsequent fermentation performance, cropped yeast condition and flocculation has been investigated in production lager and ale brewing yeast strains. The modifications in physiological state were progressive and indicated that the physiology of the crop from one fermentation affected the condition of subsequent slurries. Cropped yeast obtained from serially repitched laboratory fermentations using both wort and defined wort substitute exhibited a similar phenomenon, indicating that batch wort variation was not responsible for the deteriorating yeast condition. The reasons for the modifications in yeast condition following extended serial repitching are not known. It would appear though, that extended serial repitching does constitute a form of "repetitive stress injury" in which the yeast population is repeatedly transferred through several physiological states including exponential phase, stationary phase and stressed phase. Interestingly extended exposure to this cycle of events results in the progressive deterioration of the yeast biomass, though the extent of the effect is strain dependent.

P-55 Genetic instability in flocculation of bottom-fermenting yeast

M. Sato, J. Watari and K. Shinotsuka

In the course of studying karyotypes of yeast using Pulsed-Field Gel Electrophoresis (PFGE), we have previously reported the changes in chromosomal DNA banding patterns of bottom-fermenting yeast. Further analysis demonstrated that changes in Restriction Fragment Length Polymorphism (RFLP) of mitochondrial DNA seemed to be coincided with the chromosomal DNA changes. Our long-term study of periodic examination of brewing yeasts in our practical plants revealed that among the many brewing properties, flocculation was the most variable property; through passage of many generations in practical plants, flocculation tended to decrease gradually. In many cases, genetic alterations in the flocculation gene Lg-FLO1, governing flocculation of bottom-fermenting yeast, were detected by means of Southern- and Northern- blot analysis. It was the first time that the instability of flocculation in practical bottom-fermenting yeast was linked with the genetic alteration. Genetic instability including the changes in chromosomal and mitochondrial DNA might suggest that dynamic alterations, i.e., extensive genetic rearrangements in brewing yeast, are frequently occurring during successive generations.

P-56 Combined primary and secondary fermentation with immobilized yeast

J.H.M. Kronlof, I. Virkajarvi, E.L. Storgards, J. Londesborough and G. Dymond

Immobilized yeast is today used successfully in full scale for secondary fermentation (or aging) of beer. Two years ago we built a primary fermentation pilot plant with a capacity of 50,000 US Bushels per year. Recently we connected a corresponding secondary fermentation plant in succession to the system, forming a complete fermentation/maturation block with a total process time of two days. The system comprises of (1) a two-stage packed bed fermentation system and (2) a combined yeast separation, heat treatment and immobilized yeast secondary fermentation system. Instead of using expensive carrier materials, we introduced a low-cost material, i.e. pretreated wood chips. The price is a fraction of the price of competing materials. An extensive feasibility study showed the importance of the carrier material price on overall economy. The experiments include a comprehensive microbiological hazard analysis of critical control points (HACCP). Furthermore, cytometric methods are appraised for examining yeast physiology. Flow dynamics is monitored by means of residence time distribution tests. Most importantly, production tests are executed assessing both the efficiency of the process and the analytical and sensory quality of the final beer. Statistical methods such as primary component analysis (PCA) will be applied in the optimization of the process. The results generate a firm basis for evaluating a full-scale process based on immobilized yeast.

P-57 Optimisation of free amino nitrogen requirement of yeast.

A. Cameron-Clarke, G. Hulse and B. C. Axcell

A comparison of free amino nitrogen (FAN) requirement by a brewery strain of *Saccharomyces cerevisiae* has been carried out on freshly cropped and artificially aged yeast, as well as yeast that had been through different numbers of fermentation cycles. In addition, both maltose adjunct and dextrose adjunct type worts of varying FAN levels were used. Differences in fermentation and flavour parameters, as well as in yeast quality at the end of fermentation, were determined. In all cases, rates of fermentation depended on growth, which in turn was dependent on FAN levels. At constant oxygen charge the percent of FAN taken up decreased with increasing FAN levels. Yeast that had been through a greater number of fermentation cycles appeared to have higher FAN requirements and to take up a marginally greater proportion of

available FAN than yeast that had been through one fermentation cycle only. Other parameters that were dependent on FAN levels were final pH of the fermented wort, ester production and glycogen and trehalose levels of yeast at the end of fermentation. Fermentative capacity of yeast, as well as flavour profile of beer produced at each FAN level varied depending of whether the yeast had been freshly cropped or artificially aged. The number of cycles of fermentation that the yeast had been through also affected performance.

P-58 New malting quality technology for 2000: Helping maltsters "add value"

J. P. Murray and S. Chandra

In an effort to "add value" to business, international maltsters are now striving to get much closer to their brewery customer base. This means developing much more insight into their clients brands and brewing processes. The ultimate objective is to learn how to "customise" malt to deliver unique benefit in the eyes of each customer. Whereas the traditional malt "spec" has been a useful negotiating tool between maltster and brewer it is an imperfect device to meet these new, more strenuous demands. In recognising this shortfall, BRI has been actively engaged in developing new quality methods of assessing malt – as seen from a brewers perspective. The technology which will be presented will cover the impact of malt on beer sensory profile, brand drinkability, product wholesomeness and brewing process efficiency factors such as fermentability and filterability. New techniques such as reflectance spectroscopy will be discussed. A model of how brewers may wish to reflect their future demands on malt quality will be offered.

P-59 Pretreatment of pitching yeast with zinc

Behnam Taidi, B. Hoogenberg and A.I. Kennedy

Zinc is an essential nutrient for yeast growth and fermentation and is required as a cofactor for many enzymic reactions notably the conversion of acetaldehyde to alcohol by alcohol dehydrogenase. The concentration of zinc in wort is normally suboptimal for yeast nutrition and wort is routinely supplemented with this nutrient although excessive concentrations of zinc are toxic to yeast. Proteins and polyphenols together with other organic molecules in wort can act as powerful chelating agents which can bind to the supplemented zinc and make this nutrient unavailable to the yeast. The possibility of treating yeast slurries with zinc was investigated. Pitching yeast slurries contain a high concentration of yeast and a lower concentration of peptide and organic material capable of chaelating zinc. Provision of zinc to yeast slurries in storage would potentially make the nutrient more available to the yeast. The barriers to the uptake of zinc by pitching yeast are the low metabolic activity of yeast in storage. A controlled way of supplementing pitching yeast with zinc would be the addition of zinc to yeast during acid washing. The pretreatment of yeast with zinc during acid washing was compared to direct addition of zinc to wort. The influence of the timing of the addition of zinc to wort with respect to pitching was also investigated.

P-60 Oxidative stress and brewing yeast: Resistance, defence mechanisms and cellular damage

V. Martin, D. E. Quain and K. A. Smart

During the brewing process, exposure to oxidative stress may occur as a consequence of yeast propagation, storage and fermentation conditions. It is suggested that exposure to oxidants may influence yeast physiological condition and therefore subsequent fermentation performance and beer quality. Oxidative stress may be defined as the response to cellular damage generated by reactive oxygen species such as superoxide anions and hydrogen peroxide. These compounds are generated as by-products during yeast aerobic metabolism and may damage cellular macromolecules such as lipids, proteins and DNA. Cellular damage incurred as a consequence of exposure to reactive oxygen species may result in lipid peroxidation, protein carbonyl formation and DNA base modifications. Primary defences are provided by enzymes such as superoxide dismutases (SOD1 and SOD2) and catalases (CTT1 and CTA1) although other non-enzymic antioxidants may also provide protection (e.g. glutathione). Production ale and lager strains were grown in YPD, wort or defined wort substitute and were exposed to oxidants such as menadione (a generator of superoxide anions) and hydrogen peroxide to establish the influence of strain type and media composition on resistance. The relationship between oxidative stress resistance, primary antioxidant defence and quantification of cellular damage was investigated for a lager yeast strain both in laboratory and brewery samples. Resistance to oxidants, oxidative stress defence mechanisms and cellular damage were observed to be strain dependent and affected by media composition and growth phase.

P-61 Lager beer made with top fermenting yeast

Olav Vind Larsen, Henning Nielsen, Casper Moeller, Morten Johansen and Soeren Johannesen

Traditionally, lager beers are produced using a bottom fermenting yeast. Top fermenting yeast is used to produce ales, which are normally considered to be more aromatic than lagers. The main fermentation time for ales is normally shorter than for lagers due to the higher fermentation temperature, thereby making utilisation of equipment better. The present study is part of a larger investigation into rapid methods of production of lager beer. The study carried out has screened a number of top fermenting yeast strains to find a strain which will produce a low aroma beer, even when fermented at traditional ale temperature of 21 – 23 °C. The results of trial fermentations in EBC and 50 l pilot plant are presented and the advantages of the proposed process are discussed.