

EBC 2009 - Poster

P001

A rapid and simple method for analysis of bitterness in beer by FTIR spectroscopy

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Description of topic: Many consumer related quality attributes of beer like colour, gravity, alcohol content and turbidity can nowadays be quickly assessed by inline measurement allowing rapid and precise control of these parameters before product release. For bitterness of beer no reliable method exists to date that is suitable for routine inline measurement. The aim of the project was to develop an infrared spectroscopic measurement system for rapid and simple measurement of bitterness in beer.

Materials and methods for data collection: Different infrared spectroscopic measurement methods were applied for the measurement of bitterness of beer using samples with different bitterness values and spiked samples. In parallel, bitterness and iso-alpha acids were analysed using multivariate data analysis on the generated spectral data. Robustness of the method was assessed by testing samples from different production sites, beer types and production batches.

Results: NIR spectroscopy and mid infrared ATR technology showed too low sensitivity for the determination of bitterness or iso-alpha acids in a precision range which has practical relevance. Mid infrared (MIR) spectroscopic measurements performed with a liquid flow cell with a path length of 25 µm showed the best correlations with iso-alpha acid concentration and photometric determined EBU. By applying multivariate data analysis, the mean deviation of MIR spectroscopic measurement method to HPLC-method is below 1.3 mg/l iso-alpha-acids. The mean deviation of MIR spectroscopic measurement method to the photometric method is below 0.9 EBU.

Discussion: The advantage of the new infrared spectroscopic method is that it can be implemented as a routine measurement procedure with measurement time less than 2 minutes and that it can be operated very simple. No elaborate sample pre-treatment is necessary. The method has the potential for being used as a high throughput measurement method and in combination with a flow injection system as an inline measurement method for continuous process monitoring.

What is new - The application of mid infrared transmission spectroscopy for the measurement of bitterness value of beer is new. The application of multivariate data analysis methods on the spectral data results in a fast, precise and simple routine measurement method with the potential of being implemented inline for real time measurement during the production process.

P002

Cryoscopy as an analytic tool in lactic acid fermentation

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Description of topic: Lactic acid is often used in breweries for wort and mash acidification. Natural derived lactic acid is commonly utilized. Especially breweries operating in accordance to the German purity law are only allowed to use the so called "sauergut" obtained by lactic acid fermentation. To obtain optimum acidification results it is important to know the concentration of lactic acid. The decrease of the pH-value is depended on the buffer capacity of the fermented wort and thus the original gravity. So the pH-measurement is no quantitative analysis. Lactic acid can be measured quantifiable by titration and enzymatic, but also the use of cryoscopy could be a useful approach. Cryoscopy measures the freezing point depression. During lactic acid fermentation one mole of glucose is metabolized to two moles of lactic acid. Due to the resulting increase in solutes per kg of solvent the freezing point in the "sauergut" is lowered.

Materials and methods for data collection: Wort for lactic acid fermentation was produced from

commercial malt extract. Five different strains of lactobacilli were used for the fermentations. The pH-value was measured with a commercial pH meter. The concentration of lactic acid was analysed by titration, freezing point by cryoscopy.

Results: The pH-value correlated excellent with the amount of lactic acid produced by the lactobacilli. If the original gravity of the fermented wort changed the pH-value was no good indicator for the produced amount of lactic acid. Only the fermentation progress could be monitored by the pH-value, lactobacilli stopped fermenting at a pH of 3.0. The freezing point depression correlated with the amount of lactic acid measured by titration. This correlation was independent of the original gravity at the beginning of the fermentation process.

Discussion: This work showed that cryoscopy is a good analytic tool to analyze the amount of lactic acid in "sauergut". The advantage of cryoscopy compared to the measurement of the pH-value is the independency of a constant original gravity.

What is new - The aim of this work was to investigate if cryoscopy is an adequate analytical tool for quantitative analysis of lactic acid in "sauergut". The freezing point depression correlates extremely well with the increase of lactic acid.

P003

Loop-mediated isothermal amplification to detect and identify beer spoilage *Lactobacillus* sp. bacteria

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Description of topic: Contamination of beer by lactic acid bacteria e.g. the genus *Lactobacillus* is a major concern in the brewing industry. However, detection using culturing, conventional taxonomic tests, and existing molecular techniques are time consuming and/or costly. Recently, loop-mediated isothermal amplification (LAMP) has been reported as a simple, rapid, specific and cost-effective nucleic acid amplification method. We applied LAMP to detect and identify the beer spoilage *Lactobacillus* sp. bacteria.

Materials and methods for data collection: For the specific identification of *Lactobacillus brevis*, *L. lindneri*, *L. backi*, and *L. paracollinoides*, we developed LAMP primer sets that amplify target sequences in the 16S rRNA gene or the spacer region. To identify beer spoilage strains in the species, we also developed a LAMP primer set to detect a highly specific genetic marker. The specificity of the primer sets was assessed with genome DNA from several strains of lactic acid bacteria and isolates from breweries. And detection limit of the LAMP method using the developed primer sets was tested.

Results: The LAMP primers distinguished the target species or beer spoilage strains from other lactic acid bacteria in 40 - 60 min. Moreover, the LAMP method with these primer sets could detect about 1×10^2 cfu of lactic acid bacteria from suspensions in distilled water and beer that contained large amounts of bottom fermenting yeast cells.

Discussion: The LAMP method we developed for the *Lactobacillus* sp. bacteria specifically detected cells of the target species. The LAMP method has many advantages. It is highly sensitive, rapid, has low initial costs and fewer operational steps. We believe that this LAMP method for the detection and identification of the *Lactobacillus* sp. bacteria will be very useful for quality control in breweries.

What is new - We confirmed that the detection limit of the LAMP method in beer was almost the same as that for a suspension in distilled water. As well, the presence of the *Saccharomyces* yeasts did not affect specific amplification of the target DNA. Thus the LAMP method may be an efficient method for the direct detection and identification of *Lactobacillus* sp. bacteria in samples taken during the fermentation process as well as from final products.

P004

A rapid non-destructive method for quantification of fungal infection on barley and malt kernels

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Description of topic: Barley harvest 2007 in Europe has seen the resurgence of crops highly infected by filamentous fungi. Hence the evaluation of fungal infection on barley and malt kernels by conventional optical measurement methods lacks accuracy and can be tedious. Here we are presenting a new vision system to rapidly perform this task using the VideometerLab®. This system provides a rapid colour, shape and texture measurement, ideal to analyze kernels surfaces. The principle employed is a high-intensity sphere illuminated by light emitting diodes together with a high-resolution black and white camera. The digital image is acquired in less than 10 seconds, over an 18 bands spanning ranging from the ultra-blue (430 nm) to the near-infrared (970 nm) and then analyzed by the VideometerLab® statistical analysis software.

Materials and methods for data collection: Sixty European barley samples from harvest 2004, 2007 and 2008 were collected and screened for their degree of fungal infection using the VideometerLab® equipment. In parallel these samples were analyzed for their Fusarium and hydrophobins content using real-time PCR and/or competitive ELISA. As reference samples, kernels with low content of Fusarium and hydrophobins were selected and highly infected kernels, artificially inoculated with *F. culmorum* or *F. graminearum*, were produced.

Results: A good correlation between competitive ELISA tests, real-time PCR experiments and VideometerLab® prediction was found ($R^2=0.75$). The results obtained indicate that the videometerlab® equipment can accurately evaluate the percentage of global fungal infection in kernels.

Discussion: We believe that this system will be able to discriminate between kernels infected from non-infected ones in mixed samples. This system will allow to quantify the level of infection and indirectly the level of fungal mycotoxins in barley and malt crops. This equipment has the potential of becoming an efficient tool for screening the quality of barley and malt kernels to be used in malteries and breweries.

P005

New oxidation destructive analysis (NODA)

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Description of topic: Beer color changes during storage are explained by various mechanisms. Usually, enzymatic as well as non-enzymatic oxidations of polyphenols and/or formation of melanoidin substances are considered. The color is described through beer absorption spectrum in visible region. On the other hand reducing environment is typical for beer but it cannot explain why beer undergoes oxidation under anaerobic condition. The explanation of the paradox is the aim of this poster.

Materials and methods for data collection: Reductones, caramels, melanoidins and oxidised polyphenols were used as components of model oxidation reduction system under aerobic and anaerobic condition. Physical activators of ageing comprised heating, exposure to visible as well as UV region. Various redox indicators were used for study of oxidation-reduction state of beer or model solution. Differential spectroscopy was also applied.

Results: Yellow and brown pigments were formed by air oxidation of single polyphenols as well as by thermal degradation or alkaline decomposition of sugars. The formation of pigments derived from sugars continues at anaerobic ageing while their degradation starts under aerobic condition. The pigments represented typical acid/alkaline indicators that gradually became irreversible by various mechanisms. The natural changes of beer absorption spectra were similar to those obtained by physical and chemical activators.

Discussion: Differential spectra proved that the beer heating, oxidation by various agents or exposure to visible light showed common features associated with reductones and melanoidins polymerization, degradation or reduced pigments reoxidation. Oxidation and reduction pairs in beer slowly exchange electrons during ageing under anaerobic condition which is amplified by the oxidation agents including oxygen addition. The formation of oxidative radicals requires a reducing compound which acts concurrently as a radical scavenger. Reducing compounds can therefore show pro- and antioxidant attributes.

What is new - The collection of new procedures was created to study beer attributes and recognize the role of various groups of reducing compounds during ageing. Classical oxidation destructive analysis (ODA) used various inorganic reagents to recognize undesirable effect to beer but new method (NODA) combines ODA with physical influences such as heating and exposure to visible or UV light together with differential spectroscopy. The procedure enables better prediction of beer stability.

P006

Sorghum: An underestimated source of antioxidants and healthy polyphenols for gluten-free beer production

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Description of topic: The increasing interest of using sorghum by European brewers is due to its advantage of being gluten-free. For a long time, sorghum is also known to contain very high levels of several polyphenols (1), allowing maintaining low trans-2-nonenal concentrations in aged beers. Moreover, two stilbenes were recently identified in red sorghum in our laboratory (2).

The aim of this work was to investigate various red and white sorghums from different regions of Burkina Faso. In order to select the most promising samples for brewers, we have compared their stilbenes, flavan-3-ols and procyanidins content, as well as their antioxidant activity and their ability to prevent cardboard off-flavour in aged beers.

Materials and methods for data collection: For polyphenol quantification, stilbenes were extracted with a mixture of ethanol/water and quantified by RP-HPLC-MS/MS-APCI(+) (3). Flavan-3-ols (monomers and oligomers) were analysed by NP-HPLC-MS/MS-ESI(-) after extraction with a mixture of acetone/water/acetic acid (4). Antioxidant activity of methanolic sorghum extracts was determined by the usual ORAC method and by using the new AAPH assay, which measures the protection carried out by the sample against linoleic acid oxidation, source of trans-2-nonenal in wort (5).

Results: Red sorghum samples revealed to exhibit 5-15 times higher antioxidant activity than pale malt and only 2-5 times lower antioxidant activity than hop pellets (6).

Some red sorghum samples also revealed exceptional in terms of stilbene concentrations, bringing more resveratrol and piceid to wort than hop.

Discussion: A good selection of red sorghum permits producing gluten-free beers with very high flavour stability. Moreover, the higher content of stilbenes in such products could be interesting for low-alcoholic beer marketing.

(1) Dicko 2006 Biotech Mol Biol Review 1,21-38;

(2) Jerkovic 2007 EBC 134,1190-1198;

(3) Callemien 2005 JAFC 53,424-429;

(4) Counet 2003 JAFC 51,6816-6822;

(5) Liégeois 2000 JAFC 48,1129-1134;

(6) Lermusieau 2001 Cerevisia 26,33-41

What is new - The much higher antioxidant activity of sorghum compared to malt permits producing beers with very high flavour stability.

P007

Characterization of volatile compounds in beer by means of comprehensive 2D-gaschromatography mass spectrometry

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Description of topic: Comprehensive bidimensional gas-chromatography (2D-GC) coupled on-line with quadrupole mass spectrometry (qMS) is an innovative technique which was applied for the unambiguous characterization of the volatile profile of beers sampled using Dynamic Headspace Solid Phase Microextraction (DH-SPME).

Materials and methods for data collection: Beer volatiles were extracted with a triphasic SPME fibre under a nitrogen flow. The SPME device was then inserted into the injection port of a Trace GCxGC coupled with a Thermo DSQ-MS. The 1st column (30 m x 0.25 mm x 0.25 µm) was coated with 5% phenyl polysiloxane; the 2nd column (1 m x 0.1 mm x 0.1 µm) was coated with 50% phenyl polysiloxane. The GC program was: 50°C (1 min); to 210°C at 4°C/min, then to 250°C at 5°C/min and finally held for 5 min. Helium was used at 0.8 mL/min. The modulation period was 3 s. The DSQ operated at a scan rate of 16 scan/s at 40-300 m/z.

Results: The 2D-GC trace shows a clear distribution of the volatiles due to the non-polar/medium polar phase combination. More polar compounds, such as sulphur, aromatic and heterocyclic compounds, were more retained in the second dimension and were thus located in the upper part of the 2D plot with respect to the less polar compounds (aliphatic esters, alcohols, organic acids, ethers, hydrocarbons). The spelt beers showed higher levels of aliphatic esters (ethyl hexanoate, ethyl octanoate, ethyl decanoate and ethyl dodecanoate) and lower contents of monoterpenes (linalol, terpineol, citronellol and nerol) and damascenone with respect to Pils and double bock beers. This profile was related to the sensory analysis showing that Pils and double bock beers were bitter, while the spelt beers had a fruity and honey flavour. This was presumably due to the typical fruity aroma of the ethyl esters.

Discussion: DH-SPME coupled to 2D-GC/qMS is a promising approach for the unambiguous separation and characterization of a complex matrix such as the aroma profile of beers and was suitable to distinguish among spelt, Pils and double bock beers.

What is new - Practically no papers have been published so far on the characterization of the volatile fraction of beers by using Comprehensive bidimensional gas-chromatography (2D-GC) coupled on-line with quadrupole mass spectrometry (qMS). This is an innovative high resolution analytical technique which can be coupled to Dynamic Headspace Solid Phase Microextraction (DH-SPME) for beer quality control and authentication. The aroma profile of Spelt, Pils and double bock beers was determined.

P008

Practical brewer's approach to near infrared spectroscopy

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Description of topic: Near infrared spectroscopy (NIRS) is an instrumental method used in agriculture and food industry for many applications. A lot of papers describe also its usage in breweries, mostly for malt analysis. Laboratory of an industrial brewery has been equipped with FT-NIR device, calibration models for malt and hops were built and the method has been used for three years with various crops of barley and hops. Current experience revealed several necessary items to be done for correct work of the calibration models. This paper discusses the appropriate approach to this method to be fully functional.

Materials and methods for data collection: NIRS: FT-NIR MPA Bruker (Bruker Optics, Germany); reference methods: EBC 4.5.1, EBC 4.3.2, EBC 4.9.3, MEBAK 3.1.4.11, MEBAK 3.1.4.5.3, EBC 7.7;

samples: malt and hops (Czech rep. 2006-2008).

Results: Experiments showed strong correlation between NIR spectra and many important quality characteristics of malt and hops. Easy handling, low sample preparation requirements and fast quality prediction of raw materials incoming into the industrial brewery made NIRS usable in everyday brewery routine. In spite of lower accuracy when compared to reference methods were the results satisfactory if the calibration models were maintained properly. The maintenance of model was tested in long-term period. Our work revealed a need of continuous update of models, not only in case of new crops, and pointed out the most important rules to reach long-term models efficiency. Several other applications in brewery are shown such as quick monitoring of main fermentation, changes of mash spectrum during mashing.

Discussion: The work confirmed correlations previously described by many authors. Not only short study, but also three-year usage brought some necessary procedures for the model maintenance. All the advantages of this fast instrumental method were proved by everyday routine in laboratory. Quick analyses contributed to better production control and replaced labour demanding and time consuming conventional analyses.

What is new - Many authors dealing with NIRS finished their studies with validation of models. Our work showed results of three crops of brewer's raw materials, used the method under conditions of an industrial brewery and showed the way, how to avoid the models failure in long-term period.

P009

New cognitions on the gushing phenomenon

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Description of topic: As the brewing industry is permanently facing problems with gushing, this research focused on the application of two acknowledged gushing tests (MEBAK) in order to cope with this problem and to better understand gushing as a mechanism in general.

Materials and methods for data collection: The Weihenstephaner and the modified Carlsberg test were used to investigate relevant influential parameters on the overfoaming amount of bottled malt extracts containing CO₂.

Results: After heating the mash up to ca. 80°C, without boiling but filtration afterwards, one malt sample that according to Weihenstephaner test exhibited a high gushing potential showed no overfoaming of the carbonized wort. Although the wort sample produced thereby was boiled afterwards, gushing was not observed either. When the mash was heated above 80°C, gushing of the produced wort was increasingly induced in dependence of increased heating time. Reduced amounts of ground malt grain down to 10% did not cause gushing any more. After extended boiling applied for this 10%-standard malt, overfoaming amount did also not increase. Therefore a formation of substances relevant for gushing could not be confirmed. Gushing amount reduced significantly by inducing CO₂ bubbles in gushing wort and may be effective to reduce gushing. After bubbling CO₂ through gushing wort and apple spritzer, a lower surface tension of the upper liquid/foam segment than of the middle was observed. A higher number of minimal particles (>1 nm) were detected in non-alcoholic gushing syrups compared with the non gushing ones. Hydrophobins (from *Fusarium culmorum*), proven to have gushing characteristics, were detected as nanoparticles.

Discussion: Surface active substances (minimal particle size, narrow >1 nm) gain in importance for gushing. Bubbling CO₂ through gushing liquids seems to be effective to reduce gushing, as relevant particles may be carried up to the surface which subsequently may lose their gushing encouraging ability.

What is new - Surface active substances with >1 nm in size cause gushing. Therefore, nanoparticles become more important in examining gushing characteristics. The research helped to further approach the gushing phenomenon in general.

P010

Development of a rapid method to determine hydroperoxide lyase activity in barley and malt

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Description of topic: It is widely accepted that lipid oxidation during malting and mashing causes deterioration of beer flavour. Trans-2-nonenal, the major contributor of cardboard flavour of oxidised beer is produced by two main oxidases: Lipoxygenase 1 and hydroperoxide lyase. In this study, we developed a rapid method to determine hydroperoxide lyase activity in barley and malt.

Materials and methods for data collection: Extraction of enzyme hydroperoxide from internal production or commercial hydroperoxide
UV spectrophotometric determination

Results: A rapid method was developed to determine hydroperoxide lyase activity in barley, green malt and malt. The C.V of the method was less than 10%.

Discussion: Hydroperoxide activity was determined in different malting barley varieties and malt.

What is new - A rapid method to determine hydroperoxide activity in barley and malt.

P011

New method for characterizing high-molecular protein fractions in wort using a charge analyzing system

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Description of topic: For analyzing coagulable nitrogen in wort, high molecular protein fractions need to be determined. The exact knowledge of the content of coagulable nitrogen is of importance at wort boiling in order to predict the colloidal protein stability. Currently there exist methods for this such as the Kjeldahl which are however time consuming. In this work a new method (charge titration) was developed and applied for the determination and description of coagulable nitrogen in wort.

Materials and methods for data collection: Particle charge density and streaming potential analysis. Charge Analyzing System (CAS), from AFG Analytic GmbH, Leipzig (Germany) was used for these experiments. For titration a cationic standard, 0.001 normal polydiallyldimethylammoniumchloride solution (Poly-Dadmac), was used.

Wort granulate. GM 70 (70% barley malt 30% other starch sources) from GranMalt AG, Freising (Germany).

Hop pellets. Hop variety "Hallertauer Tradition", type 45 (8.1% α -acids) from Simon H. Steiner, Hopfen, GmbH, Mainburg (Germany).

Reference analysis. The coagulable nitrogen method according to Kjeldahl (MEBAK vol. II 2.8.2).

Results: Different trials for boiling a standardized wort were conducted to describe the precipitation (coagulation) of nitrogen using the charge titration method. The streaming potential as well as the surface net charge of the particles in wort could be reproducibly measured. It was found that a distinct relationship exists between the high molecular protein on one hand and the streaming potential, or the titratable charge, on the other hand. By this new method, the influence of hop on the coagulation could be figured out. As a result, a significant reduction of the high-molecular protein content was observed.

Discussion: With this method it was possible to predict the precipitation of high-molecular nitrogen in a standardized wort sample during boiling. It was therefore possible to optimize the cooking duration during the boiling process with respect to the colloidal protein stability.

What is new - Using a standardised wort, the mechanism of precipitation of high-molecular protein during wort boiling could be described physico-chemically with the help of charge-titration method. Furthermore, the impact of hop on the physico-chemical stability and the rate of nitrogen precipitation could be characterized for the first time.

P012

Multi-parametric sensor (MEMs) for brewing control and quality assessment

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Description of topic: Complex biochemical processes such as found in the brewing industry are a challenge for traditional control techniques. Linear techniques are limited in their ability to respond to system dynamics and non-linear techniques often lack high fidelity models or real-time state information for adaptive control. A multi-parameter sensor has been developed and tested in the laboratory that can provide unique and useful information for brewing processes. The sensor is small, low cost, and can provide continuous measurement of critical fluid properties.

Materials and methods for data collection: The MEMs sensor is comprised of five sense elements:
- a resistance temperature sensor
- a conductivity sensor for dielectric analysis
- a three-electrode electrochemical sensor for detection of chemical species or contaminants
- a two-electrode sensor element for pH level
- a viscosity sensor.

The sensor suite is capable of being integrated with process equipment for continuous operation. It operates by dynamically probing the fluid by sending different stimulus signals to the sensor elements and interpreting the response.

Results: In general sensed fluid parameters are coupled. For example, some contaminants or chemical changes such as an increased level of oxidation change the fluid dielectric response and cause a change in pH. Combining the response from multiple sensor elements permits obtaining a more accurate estimate of fluid parameters and process reactions. This sensor has also been used to monitor change in food products due to microbial activity. Several laboratory studies are in progress to pursue bio-process control using the fluid sensor such as for a yeast reaction. These efforts are directed at generating real-time state information for use in model-based control.

Discussion: This new in-line sensor represents a departure from previous sensor systems which are often passive and incorporate one sensing modality plus occasionally temperature or analyze fluid samples which are extracted from the process off-line in a laboratory.

What is new - Continuously sensing fluids with a multi-element sensor provides specific and important benefits for the brewing industry. E. g. the sensor is capable of estimating the sugar content in fluids, detecting changeover from one brand to another and the transition from cleaning to production or measure chemical elements and microbial species. The sensor provides critically needed real-time process information which can insure product quality and permit using innovative advanced control techniques.

P013

A straightforward method for the determination of viscosity-inducing arabinoxylans in wort and beer

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Description of topic: In addition of the known b-glucan influence on wort and beer filtrability, high-molecular Arabinoxylans impart similar negative effects on each, wort and beer filtrability. A method for the measurement of these high-molecular arabinoxylans has been developed to help elucidate filter problems.

Materials and methods for data collection: worts are subjected to alcohol precipitation, centrifuged at 7000 g, and the precipitate is hydrolyzed by dilute sulfuric acid.

After neutralizing with calcium carbonate, an aliquot of the supernatant is lyophilized, derivatized with sylon htp and Xylose measured by GC-analysis.

Results: A very close correlation of mash filtrability, beer filtrability and arabinoxylan-content could be shown, by using exogenous b-glucanases to eliminate influence by variable b-glucan content.

The standard deviation of the method is 4.3 %

Discussion: This presented method offers a nice tool to predict filter performance and show the needed exogenous enzyme activity to correct fluctuations due to raw materials.

What is new - The combination of precipitation of only viscosity-inducing arabinoxylans with acid hydrolysis and measurement of the xylose.

Arabinoxylan is calculated by previously published xylose content in arabinoxylan.

P014

Survey of emergent mycotoxins on malting barley

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Description of topic: Contaminants, particularly mycotoxins are one of the most serious problems in food because consumers want to eat or drink a safe product. Getting a beer with a content of contaminant as low as possible is the greatest challenge facing brewers today.

Malting barley is naturally contaminated by a large number of microorganisms among them toxigenic fungi. OTA and Deoxynivalenol that have been or will be regulated in cereal and malt by European Union have been studied.

Several Fusarium-species from barley are capable of producing emerging mycotoxins Beauvericin, Eniatin B and Moniliformin.

We developed an HPLC MS/MS method to determine these emerging mycotoxins on malting barley and malt.

Analytical methods and the occurrence of these emergent field mycotoxins in malting barley will be presented.

Materials and methods for data collection: Malting barley from Harvest 2006, 2007 and 2008.

HPLC MS/MS methods

Results: A sensitive and reliable analytical HPLC MS/MS methods have been developed to determine Beauvericin, Eniatin B and moniliformin in barley and malt. The natural occurrence of these emerging mycotoxins in malting barley has been studied. Among these mycotoxins, Eniatin B was detected at the highest level. Samples from Harvest 2007 were more contaminated than harvest 2006 and 2007.

Discussion: The survey showed that these emergent mycotoxins are in malting barley. Due to modification of infection by field fungi strains, change in agricultural practice, emergent mycotoxins surveillance should be carry out in malting barley production countries.

What is new - Only a limited number of studies have been conducted to determine the concentration levels of emergent field mycotoxins in cereal. This study gave a clear view of the occurrence these mycotoxins in malting barley.

P015

Intrinsic antifungal properties of (9S,12S,13S)-9,12,13-trihydroxy-10E-octadecenoic acid (THOE) - formation induced by mechanical stress in barley and malt

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Description of topic: Plants are able to defend themselves by different strategies like physical barriers as the cuticle or they release toxic compounds against mechanical damage, pathogens etc. The 9-Lipoxygenase (LOX) pathway leads to the formation of a 9-hydroperoxide of linoleic acid - a precursor of flavor active 2E-nonenal. In addition, LOX pathways lead to the formation of anti-fungal active agents.

Materials and methods for data collection: The determination of in vitro antifungal susceptibility was performed sterile as follows: Fungal strains were cultured on sporulation agar until they reached full growth. The fungal spores were suspended in water and 0.5 and 1.0 mM THOE was poured directly in a 24-well plate, filled up with 1 ml of PDA-agar. Fungal suspension was inoculated onto the agar of each well. The plates were incubated aerobically at 17°C until the negativ control reached full growth.

Results: (9S,12S,13S)-Trihydroxy-10-(E)-octadecenoic acid (THOE) inhibited the growth of *Fusarium poae*, *Fusarium tric.* and *Alternaria alternata*. *Cladosporon* sp. was effectively inhibited already at 0.5mM. No inhibition on vegetative growth of *Penicillium* sp. and *Epicoccum* sp. was detectable, THOE shows only weak inhibition on *Trichothezium* sp. The antifungal properties of THOE on *Magnaporthe grisea*, the rice blast fungus was tested by agar-diffusion-test, by inhibition of appressorium formation and germination tests. THOE did neither inhibit the vegetative growth of *M. grisea*, *F. graminearum* and *N. coryli*, nor the germination of *M. grisea* in complete media. Surprisingly, THOE showed an effect on the formation of an infection cell (appressorium) at concentrations as low as 0.075 mM (90%) decreasing with higher concentration.

Discussion: We were able to show antifungal effects of THOE on some tested fungals. In our hands THOE did not inhibit growth of *M. grisea* in contrast it affected the formation of an infection cell. These results emphasize the influence of THOE which is formed via an initial 9-lipoxygenase reaction of linoleic acid.

What is new - In the literature, (9S,12S,13S)-trihydroxy-10-(E)-octadecenoic acid (THOE) has been reported as anti fungal active compound inhibiting the growth of *Magnaporthe grisea*, the responsible fungus for rice blast disease. We have demonstrated the anti fungal activity of THOE against barley relevant fungi. In addition, the activation of *M. grisea* growth by THOE has been characterized, which is contrary to the published data.

P016

Malting process parameters evaluation by near-infrared spectroscopy in reflectance (NIR)

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Description of topic: The aim of this study is the development of a rapid method to control on-line several parameters during malting process for the prediction of the moment in which stopping the germination process and therefore the beginning of the kilning. The near-infrared spectroscopy in reflectance (NIR) is a non-destructive and rapid technique applied increasingly for food quality evaluation in recent years. This analytical method is widely used in brewery industry as a quality assurance for raw materials, intermediate and end products.

Materials and methods for data collection: Samples of six varieties of barley grown in central Italy were malted. The malting process was conducted by a 16 x 0.5 kg micro-malting pilot plant. The malts were kilned using a specific program appropriate for pilsner malt (final temperature of 82°C) and then analysed by means of standard A-EBC methods. The FT-NIR absorption spectra were acquired on green malt at the end of germination, before kilning. The calibration models were obtained applying a multivariate PLS regression between the FT-NIR absorption spectra of green malts and the corresponding analytical data.

Results: Various correlations were found between NIR spectra and the corresponding analytical data.

The calibration models developed were valued with cross-validation (leave-one-out) and considered satisfactory. In particular, it was possible to evaluate with a low error of prediction (aprox. 5%) moisture, total nitrogen, extract, Kolbach Index, friability, and α -free-amino nitrogen (FAN) in green malt, giving the opportunity to control on-line these important malting parameters.

Discussion: These calibration curves can be used for monitoring changes in grain composition and physical characteristics during the malting process. The data obtained constitute a preliminary study. The calibration curves must be improved with more spectra acquired on barley samples at the various stages of malting process.

What is new - An online method to predict during the germination the potential quality parameters that the malt could have at the end of kilning process can be useful for the malting industry giving the possibility to change the malting parameters (time, temperature) along with the evolution of the germination process.

P017

Evaluation of malt quality by near-infrared spectroscopy in reflectance

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Description of topic: The purpose of this study is the development of NIR calibrations to allow a rapid and easy evaluation of some important chemical and physical parameters for the evaluation of the malt quality that is useful for breweries. The near-infrared spectroscopy in reflectance (NIR) is a non-destructive, low cost, rapid and effective technology for predicting simultaneously multiple components in food products. This analytical technique is considered suitable to be applied to beer production chain, in particular, for analysis of raw materials, intermediates and end products, and in process control.

Materials and methods for data collection: The calibration models were obtained applying a multivariate PLS regression between the FT-NIR absorption spectra of the finely ground cereals (0.2 mm) and the data obtained by means of standard A-EBC methods. Different types of malt samples (pilsner, Munich, coloured and caramel) were supplied from several European malthouses, and are representative of the malts used by Italian breweries.

Results: The calibration models were verified with cross-validation (leave-one-out) and valued as satisfactory, considering their low values of Root Mean Square Error of Cross Validation (RMSECV). In fact, it is possible to evaluate various parameters on different types of malts with a low error of prediction with these calibrations curves with a low RMSECV, less than 1% for extract, coarse extract and fermentability, and less than 5% for soluble proteins, Hartong extract at 45°C, Kolbach Index, friability, and FAN.

Discussion: The methods developed can be used to measure directly from ground samples the most relevant parameters which determine the quality of malt: soluble proteins, extract, Hartong extract, coarse extract, Kolbach Index, friability, fermentability and α -free-amino nitrogen (FAN). The feasibility of NIR calibration curves and error of prediction are suitable for the use of this proposed method in quality control for brewing industries.

What is new - The development of the rapid and not-destructive method proposed in this research and the results obtained can be useful to evaluate various parameters indicative of malt quality directly from ground samples. This could be an important tool for the brewing industry in order to control the brewing process as an alternative to the official methods that are not rapid and expensive to carry on in terms of time and reagents.

P018

Correlation study between β -glucans and viscosity during wort production in a pilot-scale brewhouse

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Description of topic: The non-starch polysaccharides of barley consist primarily of arabinoxylan, β -glucans, and cellulose. β -glucan polymers originating from barley endosperm cell walls are still present in barley malt used for beer production. β -glucans have been recognized to contribute to wort and beer viscosity, foam stability, body and fullness, haze formation. The presence of β -glucans in malt affects wort and beer viscosity and brewhouse extract yield. Undegraded cell wall polysaccharides have been connected by many authors with lautering and beer filtration problems. These behaviour have been attributed to the ability of these polymers to form viscous solutions and gels.

Materials and methods for data collection: Several brewing trials were carried out in a pilot-scale brewhouse producing 110 litres of wort and using a generic lager beer recipe. Different samples of wort were analysed at different stages of wort production (first wort, sweet wort, boiled wort and whirlpool wort) to evaluate their viscosity and the β -glucans content. Viscosity was determined by an automated micro-viscosimeter. The amount of β -glucans in wort was determined with a β -glucan test kit.

Results: The results obtained shown a wide range of β -glucan concentration, with a range from 78 to 240 mg/l. The highest concentrations were found in the first wort and decreased during the following phases. Viscosity was determined at 20°C, with a range from 1.63 to 1.94. β -glucans content was correlated with viscosity for each wort sample.

Discussion: During this study, the concentrations of β -glucans in several stages of wort production were evaluated and correlated with the trend of viscosity value, to determine the release and breakdown of β -glucans during mashing and lautering. A correlation between β -glucans and viscosity was found, while the factors that caused such a wide range of β -glucans concentration in the same types of sample could be correlated with the shear forces in the mashing vessels.

What is new - The release and breakdown of β -glucans during mashing is today regarded by the brewing industries as an important stage in the control of brewhouse operations such extract development and wort separation. Therefore, the correlation between β -glucans and viscosity in different stages of the mashing process was investigated in order to understand and to promote β -glucan polymers breakdown in the brewhouse.

P019

Predictability and optimization of PVPP-stabilization in beer using anthocyanogenes, polyphenols and total oxygen content figures by means of a neural network

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Description of topic: The optimal utilization of filter runtimes is an important cost factor of beer filtration. A limiting factor of filtration batches is the capacity of the following PVPP-stabilization. The number of cold days out of the forcing test (used at Karlsberg Brewery) determine the minimal dosage of PVPP. The results of these tests last up to 3 weeks, which limits immediate and quick reactions. The aim was to adapt the PVPP dosage as far and quick as possible using the existing analyses methods. For this purpose a neural network was fed with the analytical data and compared with the results of the forcing test.

Materials and methods for data collection: Filtration runs with following PVPP-dosages: 0; 25; 30; 40; and 50 g/hl.

Analyses:

Overall polyphenols according to MEBAK II 2.21.1;

Anthocyanogenes using Skalar 5100;
Total oxygen content in the bottled beer;
Forcing test modified by Karlsberg: 3 warm days 50°C, 1 cold day 0°C, 2 warm days 50° C, 1 cold day 0°C (revolving).
Number of cold days when 2 EBC Units are reached;
Neural network.

Results: The results showed, that the number of anticipated cold days out of the neural network correlates with the measured cold days with a deviation of 9.18% on average. At this step only the anthocyanogenes and the polyphenols content was considered. Looking at the results including the total oxygen content no improvement of the statistical accuracy was cognizable.

Discussion: The linkage of the analysed data by means of the neural network with the data of the forcing test shows a linear relation and gives the opportunity of predicting the beer stability to a sufficient extent. For further improvement in the prediction accuracy more research is necessary, as the influence of the protein content in beer was not considered in this work. Practically we were able to reduce the PVPP dosage by 40% allowing a synchronized run time between the kieselguhr and the PVPP filters.

P020

Meura's laboratory mash filtration test

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Description of topic: Conventional analysis are universally used for malt purchasing specifications, these cover qualitative parameters but do not allow prediction of process ability and real brewhouse performances, especially for mash filtration, which stay a critical step of beer production. The Meura's laboratory mash filtration test allows predicting the industrial mash filterability and calculating the throw for the scale up in industrial 2001 filters.

Materials and methods for data collection: Lab mash filtration is carried out at constant pressure in a vertical filter vessel on a cloth similar to the cloth of the industrial Meura 2001 filter.

Times and volumes are recorded allowing the calculation of the mash filterability coefficient F_k .

This method has been shown to correlate with first wort filtration time on an industrial filter.

Also the Meura 2001 mash filter is known for its flexibility / ability to handle different kind of cereals.

One of the targets of this method is to help at calculation of the throw in our industrial mash filter. From cake's height measurement after filtration, the malt equivalent ratio of the different raw materials can be calculated.

Recently, an evolved version of the Meura's bomb filter has been developed. The addition of a pressurization system simulates compression steps in an industrial 2001 filter and allows determination of the compressibility of each specific cereal or combination of cereals.

Results: Years ago, Meura developed its own laboratory method for evaluation of mash filterability on a Meura 2001 mash filter. Many trials demonstrated a good correlation between lab and industrial results.

Discussion: The Meura's bomb mash filter is a useful tool in breweries equipped with a thinbed membrane mash filter.

It allows evaluation of industrial mash filterability (trouble shooting on development of a new brewing recipe), as well as calculation of malt equivalent and compressibility ratios of any cereal or mixture of cereals in use in a Meura 2001 mash filter.

P021

Use of a copper coil as a faster alternative to traditional conversion of vicinal diketones precursors

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Description of topic: The diacetyl content is an important parameter to evaluate wort fermentation. When it lowers to a predetermined value - the "diacetyl rest" - yeast should be progressively removed starting the clarification process.

During the fermentation of beer, vicinal diketones (mainly diacetyl and pentane-2,3-dione) are essentially in the form of precursors (α -acetolactic and α -acetohydroxybutyric acids). Therefore any analytical methodology aiming all potential vicinal diketones should include a preliminary step of conversion of such precursors. Traditional conversion step consists of an aeration and treatment at 60°C for 90 minutes. We present a faster alternative by means of a Copper (Cu) Coil. Beer passes through the coil while Cu acts as catalyst significantly speeding the conversion process.

Materials and methods for data collection: The sample passes through a copper coil (flow 1 mL/min) and the vicinal diketones obtained are compared with the traditional method (90 min at 60°C). Detection was made by the by following methods GC-ECD, HPLC-UV and Voltammetry.

GC-ECD: According to Analytica-EBC Method 9.24.2;

HPLC-UV: acetonitrile / acetate buffer 0.04M pH=5 (50:50), 315 nm (derivatization with o-phenylenediamine);

Voltammetry: HMDE, phosphate buffer, 0.1 M pH=7, 0.01% o-phenylenediamine, -0.95 to -0.6 V, SWV 350 Hz.

Results: Our results show that the conversion with the copper coil has similar results to traditional methodology. Furthermore, obtained results show a signal increase with temperature and the length of the copper coil. Taking this into account is preferable to use a long coil with low temperatures to avoid Maillard reactions. A good choice seems to be is an 1 meter coil at the constant temperature of 60°C.

Discussion: Copper seems to be a good catalyst for the rapid conversion of α -acetolactic and α -acetohydroxybutyric acids to α -diketones.

What is new - This work presents an innovative process for the rapid conversion of α -diketones precursors, it only requires about 5 minutes, considerably less than the classic 90 minutes. Also, this process is suitable for the on-line conversion of beer must samples.

P022

Application of ATP bioluminescence based methods in brewing and beer tapping

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Description of topic: This work is aimed on application possibilities of an ATP-bioluminescence based method for detection of beer spoilage microorganisms in a brewing process and beer tapping.

Materials and methods for data collection: An ATP-bioluminescence based pre-cultivation method has been developed and its reliability has been checked.

Results: Results show a good correlation between classical plates cultivation and this new method. Possibilities of using the ATP bioluminescence for yeast physiological condition assay has been compared to results from a flow-cytometry.

Discussion: The ATP bioluminescence based method is an applicable option for fast, reliable and quite low-priced microbiological control in breweries and tapping technology.

P023

Particle measurement in beverages - a new tool for a statement on Gushing

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Description of topic: Particle measurement can become a new tool in standard quality evaluation of beverages, because it may show a significant correlation to the phenomenon of Gushing. Further improvement of the predictability of Gushing can be considered very desirable for daily praxis. A measurement of particles is highly selective, has a broad spectrum and can be implemented as a close-to-process analysis.

In the future it could allow the production facility to take appropriate countermeasures in early stages of the process (if possible) and/or help to decide on the type of packaging container.

The first aim of the research work should be to verify a significant correlation of Gushing and certain particle sizes and finally to differentiate between Gushing-positive (G-pos) and Gushing-negative (G-neg) samples.

Materials and methods for data collection: G-pos and G-neg samples of different beverages (e.g. beer, fruit juices with mineral water, mineral water); analysis method for particle measurement: dynamic light scattering (distribution of different particle sizes within a sample) and light blockage (particle count).

Results: The distribution of the different particle sizes of filtrated beer correlates to G-pos samples. In the particle size measuring area of approx. 5 nm G-pos beer samples show a peak, whereas G-neg beer samples do not show any particles of that size. Furthermore the particle count-analysis method showed distinctive differences between G-pos and G-neg samples of apple juice with mineral water and mineral water in different packaging containers (glass- and PET-bottles).

Discussion: The results indicate that particle measurement can be used for final beverages regarding a differentiation between G-pos and G-neg samples. It is although not known what the Gushing-relevant particles consist of on a molecular level; this must be investigated.

In the next step of the research work we shall proceed to define, isolate and identify Gushing-relevant particles both in semi-finished products (e.g. wort, unfiltered beer) and final products.

P024

Possible use of advanced microscopical techniques to evaluate the ultra structure of grain

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Description of topic: Starch, proteins and cell wall material are the main components of grains and they determine the value and the processability of these grains for certain processes and products. In general these components are analyzed using chemical methods and this offers usually sufficient information. But especially if the aim is to understand the modifications in the grain e.g. during malting it is important to visualize this ultra structure and its changes. Scanning electron microscopy (SEM) and Confocal scanning laser microscopy (CLSM) are useful tools to determine these changes.

Materials and methods for data collection: Samples of different grains were taken and observed by SEM as well as CLSM. For SEM freeze-dried samples were cut into longitudinal sections using a razor blade. Samples were mounted on circular specimen holders with double carbon tape. A JEOL scanning electron microscope type 5510 (JEOL, Tokyo, Japan) at 3 to 5 kV was used to gather SEM micrographs. Samples for CLSM were also cut longitudinal. Thereafter they were stained using Rhodamin B, FITC and Calcofluor White and were placed onto a well slide using a needle before the application of a glass coverslip. A FV300 confocal laser-scanning system (Olympus, Germany) mounted on an Olympus IX80 inverted microscope was applied to create images.

Results: SEM makes it possible to distinguish between different types of starch granules. Furthermore, certain degradation processes of cell wall material and of starch granules by amylolytic enzymes can be visualized.

With CLSM it is possible to use specific fluorescent dyes which bind to certain components and therefore show their distribution. To a certain extent it is even possible to quantify these substances.

Discussion: SEM is a useful tool to determine changes on the ultra-structural surface since extremely high magnifications can be achieved, whereas CLSM can be effectively used to make specific biopolymers visible and with an additional effort even quantifiable.

P025

Contamination of barley with *Fusarium* species related to the occurrence of discoloured kernels, mycotoxin content and consequences for the brewing process

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Description of topic: The contamination of barley with moulds is one of the most critical quality criteria. Fungal metabolites can compromise processing and consumption. Especially the occurrence of *Fusarium* species in the raw material is associated with mycotoxin contamination and the gushing phenomenon. The *Fusarium* contamination is often assessed only by visual inspection for discoloured kernels (red/black). Our investigations relate the occurrence of discoloured kernels to the contamination with *Fusarium* species, mycotoxin content and observations during and after the malting and brewing process.

Materials and methods for data collection: Inoculated and naturally infected field samples suspicious for gushing potential have been investigated for occurrence of *Fusarium* species with cultural and molecular techniques. By visual inspection suspiciously discoloured kernels have been identified and counted. Collected discoloured kernels have been investigated for *Fusarium* species as possible cause for the discolouration.

The samples have been malted and brewed in the experimental scale. The mycotoxin contamination has been analysed by LC/MSMS at selected stages from the raw material to the final product. The LTP1 content has been investigated as a possible indicator for the gushing potential.

Results: In our investigation the results from the visual inspection for discoloured kernels could not be related to the contamination with *Fusarium* species, mycotoxin content and the occurrence of gushing.

Discussion: Our results suppose that the visual inspection for discoloured kernels is not a sufficiently reliable parameter for *Fusarium* and mycotoxin contamination of barley as the raw material for beer production. Further investigations regarding the entire production chain from the raw material to the final product are needed to understand the relationship between and find appropriate measures for identification and control of potential risks.

P026

Analytical profiling of varietal floral and spicy hop essences by SPME in combination with monodimensional and comprehensive two-dimensional GC

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Description of topic: The objective of the present study is detailed characterisation of the volatile profile of novel varietal floral and spicy hop essences. This research is part of a PhD study focusing on determination of flavour-active compounds in highly enriched hop oil fractions and on evaluation of these essences in brewing.

Materials and methods for data collection: Hop oil fractions with floral and spicy flavour characteristics, respectively, are prepared by an innovative extraction/fractionation procedure based on extraction of hop pellets by supercritical fluid extraction (SFE) with carbon dioxide and further chromatographic fractionation of the extracts by solid phase extraction. The analytical profile of the hop essences is determined by headspace solid phase microextraction (HS-SPME) combined with monodimensional (GC-MS) and comprehensive two-dimensional GC (GCxGC-TOF).

Results: As demonstrated by SPME-GC-MS floral essences mainly consist of β -myrcene, and further comprise a series of esters and ketones. Spicy essences are mainly composed of sesquiterpene

epoxides and alcohols. When compared with commercial floral and spicy hop essences, significantly different and much more specific analytical fingerprints are observed for the in-house essences, due to highly improved fractionation of hop oil. Profound characterisation of the essences was performed by two-dimensional comprehensive GC, revealing previously undetected components in both hop oil fractions.

Discussion: Detailed characterisation of the hop essences is required in view of further research on the flavour-activity of hop oil compounds. Interesting findings with regard to the novel floral essences are the presence of β -myrcene as predominant compound, and the absence of linalool and geraniol, often regarded as being responsible for floral hop aroma. The complexity of the spicy essence is demonstrated by comprehensive GC. The high resolution of GCxGC results in detection of more compounds with pure mass spectra which offers new perspective for unraveling noble spicy hop character.

What is new - - The low density carbon dioxide extraction of hop pellets resulting in highly enriched novel floral and spicy hop essences. This innovative extraction/fractionation technology is in sharp contrast to current industrial procedures for preparation of hop essences

- Application of HS-SPME combined with GC-DSQ and GCxGC-TOF on highly enriched hop essences.

P027

Characterization of reductones by ^{13}C -NMR spectroscopy

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Description of topic: Norfuranol (1), glucose reductone (2) and maltol (3) are important Maillard reaction derived reductones. In the past, their formation was investigated from several sugar / amino acid reactions; however, unequivocal pathways leading to 1, 2 and 3 are still not fully understood.

Materials and methods for data collection: In the present study, isotopomers of 1, 2 and 3 were synthesized from 1- ^{13}C - and 5- ^{13}C -D-ribose, 1- ^{13}C - and 6- ^{13}C -D-glucose and characterized by ^{13}C -NMR spectroscopy. Carbohydrates and amine compounds were heated to 120-140°C in water or buffer, extracted with pH dependence and analyzed by NMR and GC-EI/MS.

Results: The ^{13}C label position of norfuranol (1) and glucose reductone (2) can be estimated by electron impact MS spectra, the position of ^{13}C labelling in maltol (3) must be determined by NMR. In addition, the interpretation of EI-MS fragmentations is sometimes difficult and has to be supported by ^{13}C -NMR data. The labelling pattern of 1, 2 and 3 dramatically changes with the nature of the amine reaction partners of the sugar. gamma-Aminobutyric acid, a heptapeptide with high lysine content and β -casein led to differently labelled reductones after Maillard reaction with ^{13}C sugars (unpublished data). Therefore, formation pathways were elucidated by analyzing isotopomers of reductones 1, 2 and 3 by ^{13}C -NMR and EI-GC/MS.

Discussion: The results clearly show the different reactivity of amino acids, small peptides and proteins catalyzing the formation of reductone structures. Reductones are important in terms of antioxidative capacity of food products and contribute to their flavour.

What is new - Previously, the reactivity of amino acids catalyzing Maillard pathways and the formation of reductones were the focus in food chemistry. Here, we present data on the reactivity of peptides and proteins.

P028

A novel method for the detection of arabinoxylans

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Description of topic: While the effects of high molecular beta-glucans on the processability of malt have been extensively studied, comparable studies on the impact of arabinoxylans (AX) on the brewing process have yet to be performed. AX are considered a main cause for filtration and other problems in the brewhouse.

Until now, an easy-to-use and reliable detection method for AX was not available. Our project therefore focuses on the development of such a method, making it possible to distinguish between water-soluble and water-insoluble fractions or high and low molecular weight fractions, respectively.

Materials and methods for data collection: Polyclonal antibodies were generated in rabbits using purified wheat arabinoxylans. The antibodies were bound covalently to a sepharose matrix and the complex used for the enrichment and purification of AX from different solutions by immunoaffinity chromatography. After elution from the column, the concentration of AX was measured using a non-specific fluorescent dye. Another approach used a refractometer for quantification.

Results: The polyclonal antibodies proved to be specific for AX, showing no cross reaction with other polysaccharides present in wort and beer. The AX were effectively separated from other substances in malt, wort and beer by immunoaffinity chromatography. Cleaned eluates could be quantified using a fluorescent dye whose fluorescence increases linearly with AX concentrations. Quantification of AX was also possible using a refractometer.

Discussion: With the newly developed analytical method, it is possible to specifically detect arabinoxylans from malt, wort and beer in a simple and reliable way. The new method can be applied in routine analysis, as well as in research, giving new impulses for industrial quality control and plant breeding.

What is new - An easy-to-use and cost-effective method for the detection and quantification of different fractions of arabinoxylans is now available. The novel combination of immunoaffinity chromatography and a fluorescent dye enables the user to quantify arabinoxylans from a multitude of different matrices in a fast and reliable way.

P029

Fuchsin Index (FI)

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Description of topic: The flavour stability of beer is an essential quality characteristic. For prognosis and analytical evaluation of flavour stability various analysis methods have already been established (e.g. thiobarbituric acid index, absorption integral and aniline index). We present our new method.

Materials and methods for data collection: Methods used were determination of thiobarbituric acid index, aniline index, absorption integral and new method fuchsin index. The principle of fuchsin index is reaction of carbonyl compounds, sulphur dioxide and fuchsin. During this reaction violet colour appears, its intensity is measured at 550 nm.

Results: The FI correlates with ageing-related aroma substances or their precursors, formed as a result of thermal stressing of malt and wort and coming to the fore in beer ageing. For that reason, this index is important for assessment of malt and wort in terms of taste stability of beer.

Discussion: In aged beers the influence of substances already known as beer ageing indicators was evaluated for their contribution to the thiobarbituric acid index, absorption integral, aniline index and fuchsin index.

What is new - The fuchsin index was created as the alternate for thiobarbituric acid index, absorption integral and aniline index. This new method correlates well with methods mentioned above but is much faster.

P030

Determination of sulphur dioxide by flow-through chronopotentiometry and its importance for brewing practice

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Description of topic: There are many methods for the determination of total sulphur dioxide in beer. The most frequently used are the three methods recommended by the European Brewery Convention (EBC): EBC methods, 9.25.1. - Total Sulphur Dioxide in Beer: Distillation Method, 9.25.2 - Total Sulphur Dioxide in Beer: Enzymatic Method and 9.25.3 - Total Sulphur Dioxide in Beer: p-Rosaniline. Very good alternative to these methods are electrochemical methods and namely flow-through chronopotentiometry.

Materials and methods for data collection: The electrochemical determination of dissolved sulphur dioxide presented here was based on an adsorptive collection of sulphur dioxide on a gold electrode set to a suitable potential. In the next step, the deposited sulphur dioxide was electrochemically oxidised, presumably to sulphate. In the chronopotentiometric mode this oxidation was carried out with constant current whereas the potential of the electrode was monitored providing the chronopotentiometric response curve.

Results: In order to assess the quality of the proposed method, determination of total SO₂ in a set of 15 beer samples was carried out by the electrochemical method and by the EBC recommended methods mentioned above. The values of the standard deviation and repeatability limit obtained with the electrochemical method were similar to the results measured with distillation method and lower than those obtained by the enzymatic and spectrophotometric methods.

Discussion: The results obtained with the p-rosaniline method were mostly slightly higher than those obtained with the electrochemical method. Higher SO₂ levels by the p-rosaniline method have previously been reported when compared with the distillation method. The precision of the enzymatic method is not as good as that of the other methods. The results obtained with the distillation method were slightly higher than the results obtained with the electrochemical method.

What is new - The electrochemical method for determination of sulphur dioxide proved to be faster and simpler than the other methods. This predestinates the chronopotentiometric method for routine applications.

P031

Development of a FMEA (failure mode and effect analysis) as an instrument of weak-point analysis in a brewery; using the example of the keg filling plant at Karlsberg Brewery, Germany

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Description of topic: New plant components have been installed in an existing keg filling plant. The standard tool FMEA was adapted to the brewery requirements. The interaction between the units were examined and possible weak-points were detected. Target was to optimize the filling process and to improve the productivity by minimizing unexpected breaks.

Materials and methods for data collection: Prior to the FMEA checklists and questionnaires were developed. The out coming information were used to form a structure and to determine the extent of the following FMEA. This analysis was done using the know-how of all involved departments (as part

of a comprehensive teamwork).

The FMEA is split into two parts:

1. The failure analysis, in which filling reports, pareto analyses, cause and effect diagrams as well as failure matrix diagrams were used.
2. The risk analysis and risk evaluation.

Both analyses were combined, resulting in risk priority figures for each examined component, which gives an indication of the need how to manage defined failures.

Results: The execution of the FMEA led to a detailed documentation of the keg filling plant and the filling process.

Based on the risk priority figures a catalogue of necessary measures were determined, separated into four categories:

- Easy and fast implementations
- Urgent implementations with costs
- Difficult implementations
- Unrealistic solutions

Even the realization of the easy and fast implementations led to a significant improvement of key process indicators.

Discussion: FMEA allows to avoid failures. It is possible to apply it in existing processes, or even better - in a preventative way - already in the planning period of new plants.

Furthermore a FMEA is applicable in other processes e.g. in the development of new products. The advantage of the FMEA is its affinity to existing methods, especially the HACCP-analysis, which allows a comparable easy introduction and implementation in a brewery.

P032

Modified Gushing Test - Reproducible gushing analysis through optimised and harmonised analytical conditions

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Description of topic: The gushing problems, which arose after the 2007 harvest and are still ongoing, have led to animated discussions about suitable methods to determine the risk potential of the raw materials. Generally speaking, the "Modified Carlsberg Test", on the basis of mineral water, has proven its usefulness as a tool for risk assessment and handling of malts in this critical situation.

However, the insufficient reproducibility of results from different laboratories gave cause for criticism.

Materials and methods for data collection: The repeatability and the reproducibility of the gushing analysis results were scrutinised in various round-robin tests in which research laboratories as well as experienced malting and brewery laboratories took part. The existing laboratory equipment and the exact procedures used were recorded in detail and compared. Numerous variations were found to exist between the various laboratories. A modified analytical procedure was developed in order to harmonise the test conditions and the equipment in the laboratories. The optimised method was validated in round-robin tests.

Results: The results of gushing analysis of malts with a very different risk potential showed a good repeatability within a laboratory. However, in the inter-laboratory tests, the results were sometimes contradictory. The insufficient reproducibility of the results between laboratories was attributed to variations in the sample preparation, the water-matrix used and, above all, the way and the intensity of the shaking during the incubation. After an alignment of the laboratory equipment and a rigorous adherence to the detailed modified analysis procedure, significant improvements were achieved in the reproducibility of the results from further round-robin tests.

Discussion: The results presented here are an important step on the road to the establishment of the "Modified Gushing Test" as an internationally accepted standard method for the determination of the gushing risk of malts.

What is new - A modified gushing test with high repeatability and good reproducibility.

P033

Novel proteomic studies performed with UPLC chromatography and nano ESI-QTOF-MS/SMSMS: Comparison of the protein content in unhopped wort, cold wort and bright beer

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Description of topic: During the brewing process the protein content dramatically changes: wort boiling, the addition of hop, fermentation, filtration procedure and in some cases flash pasteurization exert influences, which configure the protein pattern in the bottled beer.

The protein diversity strongly decreases till the end product. Major proteins discussed with topics like foam stability, gushing or haze formation are LTP and protein Z. Tracing them throughout the brewing process and elucidation of modifications are of great interest.

In general proteomic studies are performed with gel electrophoresis and additional MALDI-analysis.

Here we present a new way to do proteomic analysis irrespectively operating from gel separations.

Materials and methods for data collection: A special sample pretreatment consisting of lyophilization and phenolic extraction was performed. The purified and preconcentrated samples were analysed for native and denatured proteins as well as for peptides after tryptic digestion.

An Acquity UPLCTM chromatography has been introduced for native and denatured proteomic studies using an Acquity UPLC[®] BEH300-C4 column (1.7µm, 2.1x150mm) and a micromass Q-TOF microTM. The chip-based Triversa Nanomate robot system was used for MS infusion in combination with protein fraction collection into well plates. Subsequently this method was upgraded for protein identification with nano ESI-MS and -MSMS (Data Directed Analysis) as the protein fractions were digested and reinfused. The method both allows for the analysis of highly complex samples as well as low concentrated ones. Special software tools help to structurally identify proteins via their peptide pattern and aminoacid-sequences.

Results: The results of this study present proteins identified in unhopped wort, cold wort and beer.

Discussion: This new technique could also be applicable to distinct research interests like foam or haze analysis.

P034

A new method of sensory evaluation

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Description of topic: Flavor is the significant criterion of evaluation in beer tasting, which depends on many primary and secondary causes. This includes desirable and undesirable aromatic compounds, but also haptic and physical issues like viscosity CO₂-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 beer of 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.

Materials and methods for data collection: For testing the method a large amount of students were tested concerning their degustation. Therefore samples of beer and beer flavored with different

concentration of an unwanted aromatic substance (Benzaldehyde) have to be tasted.

Results: The results are very good. It can be seen that the results describe a probability distribution and that the number of correctly tasting persons for every concentration can be calculated.

Discussion: A discussible point is the adaptability of the presented method for the beverage industry and its possibility for process optimization (e.g. influence of a new aggregate in the brew house concerning the taste of the final beer).

What is new - The new sensory evaluation method is an excellent tool for determining the relation between changes in a product and the acceptance of the consumer. 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.

P035

Improvement of the evaluation of the mashing conversion yield and the sparging efficiency

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Description of topic: Conventional methods of the determination of extract losses in the form of spent grain extraction are not suitable in many cases. Both (percental) numbers are related to the absolute amount of spent grains, which is not known in practice. The mass of the spent grains can differ considerably. The new method determines the spent grain amount by a mass balance of the extract. Laboratory mashing equipment simulates the large scale process. A model describes the fate of the extract during mashing and lautering. Herewith it is possible to determine the conversion yield of mashing and the mass of the spent grains. The model was verified by gravimetric measurement of the spent grains amount. With the knowledge of the spent grains mass the soluble and convertible spent grain extract can be converted into the actual absolute losses of extract.

Materials and methods for data collection:

- Standard laboratory equipment (laboratory mills, plan sifter, mashing equipment, drying oven, soxteth extraction device, density measurement).
- Mashers different in malt quality, grist fineness and mashing intensity
- The conversion yield was measured and calculated from the extract concentration (liquid and solid phase of the mash).
- Mashing conversion yield was derived with the help of a simple mathematical model.
- Accompaniment by investigations in various breweries.

Results:

- Depending on the process parameters (malt, grist, mashing) remarkable differences in the conversion yield, justified by convertible spent grain extract.
- Improvement of conventional spent grain extract analyses can be improved with the knowledge of the spent grains mass.
- With the new method the spent grain mass can be estimated quite precise.
- Conversion yield is a new size to distinguish conversion yield from sparging efficiency and it correlates with the convertible spent grain extract.

Discussion: The Proposal bases on spent grain extract analyses. A more exact and distinctive comparison is possible.

What is new - • the direct determination of the conversion yield instead of indirect of convertible spent grain extract.

- the determination of the absolute spent grain mass.
- the total concept for tracing the fate of extract
- the necessity for this work because the decline of total wort boiling evaporation and changes in malt prices in the recent years let brewers place more attention at the extract yield and the required sparging liquor amount.

P036

Spoilage yeasts in breweries and their detection by realtime multiplex PCR

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Description of topic: Spoilage yeasts are a everlasting danger for beer and beer mixed beverages and the hygiene of their production surroundings. Saccharomyces belong to the most dangerous spoilage yeasts e.g. S. diastolicus. Most non-Saccharomyces spoilage show lower fermentation capacity. Most of them are only able to grow if fermentable sugars and oxygen are available in the beer or beer mixed beverage. A recent study shows that the non-Saccharomyces species Pichia anomala is a dominating contaminant in the filling area and is biofilm forming. In this study the yeast distribution in 106 contaminated beer samples was observed with a focus on Saccharomyces spp. and Pichia anomala and a specific multiplex realtime PCR kit was used for the analysis.

Materials and methods for data collection: Contaminated beer samples were received from BLQ Weihenstephan (TU München) The beer samples were filtered, incubated on wortagar and single colonies were identified by 26S rDNA-sequencing. The beer spoilage potential yeasts strains were inoculated into pasteurized beer and alcohol free beer and incubated for 14 days at 28°C. To analyse yeasts growth the turbidity of the inoculated beers was checked visually. The yeast DNA is extracted and the used realtime multiplex PCR kit identifies Saccharomyces spp., Dekkera spp. and Pichia anomala in one single test.

Results: In this study 106 yeasts strains were isolated from beer and identified on species level by 26S rDNA-sequencing. The percentage of the Saccharomyces sp. was 14,2% and the percentage of Pichia anomala was 17,9%. The strains of Saccharomyces sp. and Pichia anomala were inoculated in beer and alcohol-free beer and incubated for 14 days at 28°. All strains showed beer spoilage potential after 14 days. All strains of Saccharomyces sp. grew within 4 days. On genetic level the yeasts are detected and differentiated by multiplex realtime PCR.

Discussion: The study shows Saccharomyces sp. and Pichia anomala as the most frequent yeasts occurred in breweries which can be excellent identified by using realtime PCR.

P037

Carrageenan - an effective tool for wort clarification in the brewhouse (laboratory vs industrial trials)

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Description of topic: Carrageenan makes an effective tool allowing to obtain a clear wort in the brewhouse. It enhances natural sedimentation of haze particles present in wort. It's addition causes a rapid clarification of wort on cooling, giving a better start to stabilize beer right at the beginning of the production process.

Materials and methods for data collection: Commercially available carrageenan (Promar, Warsaw) was added to the boiling wort of 9 Plato (lab and brewery) 18P (lab trials) 10 min prior to the end of boil at various rates (0, 50, 100, 200 mg/L). The hot wort was left to cool (laboratory stage) or was cooled in a heat exchanger (commercial brewery - 10hL boil). The wort haze, color, pH, extract, total protein and some metal ions (Zn, Mg, Ca) was analyzed. Additionally the green beer was analyzed for haze and a visual observation of the cropped yeast quality was assessed (photos presented).

Results: The clarity of wort significantly increased, proportionally to the amount of carr. added, as well as the amount of precipitate (photos presented). The optimal value seems to be 50 ppm of carr. (minimum amount of precipitate with a satisfactory level of haze). Protein content dropped by ca. 25% when 100 ppm carr. was added, while further increase of its addition did not influence the protein

content. The use of carr. does not influence the analysed microelements concentration in wort. The influence of carr. addition on wort clarity is wort density dependent - for the weak wort (9P) the highest value of carr. (200 ppm) caused an increase of haze as compared to the control. Whereas in the case of stronger wort (18P) such influence was not noted.

Discussion: The clarification of wort at the stage of boiling decreases its haze by 50-80% depending on wort concentration. This reduces the amount of particles which may stick to the yeast cells, making the cropped biomass much clearer. The clarification of wort at the early stage (brewhouse) may enhance the length of beer filtration runs allowing for a reduction of diatomaceous earth usage.

What is new - The use of carragenean was assessed both for laboratory and industrial scale. Photos of cropped yeast in the fermentation tank were taken proving a much better quality of yeast obtained after the fermentation of clarified wort. The comparison of wort clarity was also shown with the use of camera, which also allowed to reveal how the amount of precipitate increases (after cooling the wort in a cylinder). The results presented in a illustrative was and shall be interesting for the brewers.

P038

Haze-related phenolic structures generated through beer aging. Proposal of a sensitive colloidal instability assay using microwells

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Description of topic: Beer composition (flavour, colour, haze...) changes through storage, thereby altering the quality of the product. Beer flavour is known to be deteriorated due to the release of compounds like T2N (1), DMTS (2) or 4-VG (3). Recently Dehydrodicatchin A with a yellow brown colour has been identified as responsible for beer colour modification through aging (4). Very little accurate information is available regarding the phenolic structures involved in haze formation. Yet these data are required to propose new colloidal stability assays giving better prediction for brewers.

Materials and methods for data collection: Increasing concentrations of oxidised polyphenolic structures (isolated from model media by semi-preparative HPLC) were analysed in microwell in presence of gelatin. The decrease of transmittance was monitored at 680 nm (5). The most interesting fractions were further identified by HPLC-ESI-MS/MS and quantified in aged beers.

Results: Various model media of catechin and procyanidins were monitored in order to mimic beer storage. After isolation of the degradation products and identification by RP-HPLC-ESI(-)-MS/MS, their haze potential was measured. New compounds derived from dimer oxidation revealed very potent to interact with proline-rich proteins. A new assay using microwells is therefore proposed to accurately quantify the haze polyphenolic fraction of a beer.

Discussion: New assay using transmittance measurement in microwell allows to quantify the tanning capacity of a beer. For the first time, the structures of a polyphenolic fractions involved in colloidal haze are determined.

(1) Noël et al., J. Agric. Food Chem., 47 (10), 1999, 4323-4326 ;

(2) Gijs et al., J. Agric. Food Chem., 50, 2002, 5612-5616 ;

(3) Callemien et al., J. Agric. Food Chem., 54, 2006, 1409-1413 ;

(4) Callemien and Collin, J. Agric. Food Chem. 2007, 55, 9066-9073;

(5) Poupard 2008.

What is new - For the first time, the structures of a polyphenolic fractions involved in colloidal haze are determined.

P039

Relationship of temperature, particle size and clarification - an investigation in haze stability of

wheat beer

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Description of topic: Stable haze is an important quality aspect of German "Hefe-Weizenbier" (top fermented wheat malt beer). Haze in this beer is caused primarily by yeast and colloids. The progress of haze according to different storage temperatures (4 °C and 20 °C) were monitored nephelometrically and by detecting the particle size distribution in the supernatant of 0.5 L bottles at a measurement temperature of 12 °C over 8 weeks.

Materials and methods for data collection: 10 German wheat beers in 0.5 Liter bottles, stored at 18-20 °C;

Thoma chamber for yeast cell counting;

Haze measurement was done with Sigrist "Labscat", 90°/25°-angle scatter light intensity Sympatec "Helos" particle sizer with Sympatec "WINDOX 5" software.

Particle size distribution was measured with 500 ml (2 x 250 ml supernatant of 0.5 Liter bottles) of wheat beer at a temperature of 12 °C.

Results: The results show a high sedimentation rate of yeast in cold and warm stored samples, due to its diameter $x > 3 \mu\text{m}$ over the time of 8 weeks. The availability of colloids as haze effecting particles (at 12 °C) was strongly influenced by the storage temperature and the resulting physical phase due to the temperature. Both samples showed a bimodal distribution. The cold stored samples show a high amount of precipitated colloids, accompanying a sedimentation characteristic like solid particles, the residual particles have had a diameter of 1 μm . Samples stored at 20 °C showed almost no precipitation and have had a similar intensity of turbidity and particle size distribution ($x < 4 \mu\text{m}$) at the point of measurement as initial.

Discussion: The results show the influence of temperature on colloids in beer and confirm the influence of chilling and its effect on clarification in the cold stored samples, with residual particles with a diameter close to 1 μm . The effect of Brownian motion on agglomeration and precipitation of colloids in unfiltered beer seems to be negligible and the residual particles can be up to 4 μm .

What is new - This investigation monitors agglomeration, precipitation and sedimentation in unfiltered beer over time by particle size distribution with an approach to characterize beer as a colloidal multi substrate solution. Due to this, investigations based on particle size measurement can be useful to scrutinize different problems of brewing like filtration problems and new formation of haze in filtered beers.

P040

Gushing malt leads it to gushing beer?

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Description of topic: Gushing is one of the most serious beer faults. Gushing tests have been carried out directly on malt by extracting gushing factors from malt followed by introducing the factors to a beer or sparkling water. An ELISA method to detect Hydrophobins, a small surface active proteins produced by filamentous, was also developed to predict the gushing risk of malt. The importance of these methods on malt to predict the gushing risk of beer is controversially. The aim of this investigation was to study at the 20 hl brewing scale, the impact of gushing malt to gushing beer.

Materials and methods for data collection: Brewing of pils beer at 20 hl scale with commercial and pilot malts having various level of gushing.

Gushing of malt was determined by different methods. Mycotoxins of malt were determined by HPLC MS/MS.

Gushing of beer was determined after 3 days of rotation.

Results: The present study at 20 Hl brewing scale showed that gushing positively malt have no led to gushing beer. Gushing inducers were followed during brewing process to know at what steps, these were loosed.

Discussion: The gushing methods on malt is not a useful tool to predict the gushing risk of beer.

What is new - Beer brewed at 20 hl scale with malts having various level of gushing.

P041

Upstream beer stabilisation during wort boiling by addition of gallotannins and/or PVPP

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Description of topic: Haze development in beer during the shelf life is a persistent problem in the brewing industry. Pilot-scale brewing trials (12°P) were conducted to study the effect of gallotannins and PVPP addition in the boiling kettle on colloidal and flavour stability, this in combination with pH control.

Materials and methods for data collection: Brewing trials (50 L) were conducted with gallotannins and PVPP addition at the end of the wort boiling. The influence of pH was studied by mashing in at 5.6 and 5.2. After oxygen-free conditioning, the beers were evaluated analytically. Total polyphenols and flavanoids were measured according to EBC methods. The content of proanthocyanidins was detected with the method of 'Bate-Smith'. Sensitive proteins were detected by the IOB-method 9.37. Chill haze (25/90) was measured after a 24 hour storage at 0 °C. Residual gallic acid was analysed by HPLC. Reducing power was determined by the TRAP and DPPH test. Sensory evaluation was performed by a taste panel.

Results: Brewing trials in the presence of gallotannins showed an efficient removal of sensitive proteins with only a small increase of the gallic acid content and a positive influence on taste and reducing capacity of the fresh beer. The evolution of chill haze related to the aging process showed that beers stabilised with 10 g/hL gallotannins in combination with pH correction gave the best results. The addition of 10 g/hL PVPP has an explicit effect on the amounts of polyphenols, which results in an improvement of the colloidal stability. The use of both stabilisation agents during wort boiling showed a decrease of the sensitive proteins and the haze-forming polyphenols. This in combination with an increase of the reducing capacity.

Discussion: The use of gallotannins and PVPP upstream in the brewing process has a positive impact on the shelf life of beer. Application of appropriate stabilisation products during boiling is a very convenient way of physicochemical stabilisation without the need for extra filtration and/or beer losses.

What is new - The addition of PVPP or gallotannins at the end of the wort boiling in the kettle results in a positive evolution of the colloidal stability, by bonding of the stabilising agents with the sensitive polyphenols or the sensitive proteins. Sequential addition of gallotannins and PVPP at the end of wort boiling reveals an important increase of the shelf life.

P042

Effect of surfactants on beer foam stability and collapse

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Description of topic: Beers of different composition were tested experimentally, as for the effect of organic compounds with surface activity on the dynamics of beer foam stability and collapse. The

high-speed imaging system was used to monitor the foam behaviour, at high resolution.

Materials and methods for data collection: Several compounds were used to study their effect on the beer foaming system (proteins, iso- α -acids, polyphenols). The liquid phase was a set of samples of beers as well as beers after addition of surface active compounds. Gas bubbles (CO₂, N₂) were produced with help of fine porous spargers. The foam dynamics was recorded visually (Photron digital camera). The kinetics of the foam growth and decay was evaluated and related to liquid properties (viscosity, surface tension, dilatational modulus).

Results: The temporal pattern of the beer foam behaviour was obtained in terms of the kinetic parameters and their relation to the character of the surface active compound.

Discussion: The results are promising in relating the foam kinetics and the chemical structure of the surfactant.

What is new - New data on the link between the macroscale foam behaviour and the chemistry of surfactants.

P043

High-speed imaging of beer foam formation: Effect of surfactants

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Description of topic: In this experimental study we developed a visualization method based on the high-speed imaging technique. The method consists of application of high-speed digital camera and image analyzing software. The method is suitable for investigating the mechanism of bubble formation (heterogeneous nucleation) and collision of bubbles with liquid surface. The suppression of bubble coalescence, whence foam formation, was tested on different samples of model liquids and beers differing in surfactant content.

Materials and methods for data collection: Several surfactants (proteins, iso- α -acids, polyphenols) were tested in laboratory experiments, under well-defined conditions. Gas bubbles (CO₂, N₂) were introduced into liquid phase in a controlled way by a bubble generator and the collision of bubbles with gas-liquid interface was studied with a high-speed digital camera (Photron) and image analyzing software (MathLab). The rheological properties of these model liquids were characterized by traditional parameters (density, viscosity, surface tension) as well as by dilatational modulus, a parameter characterizing the dynamic properties of foam.

Results: We obtained interesting relations between the flexibility of the liquid film surrounding the gas bubbles and the composition of the liquid phase. The influence of surfactants on mechanism of bubble formation and their collision with gas-liquid interface was studied.

Discussion: The results indicate the relevance of the presence of various kinds of surfactants on the foam properties.

What is new - The fast imaging method for investigation of the foam formation affected by surfactants on short time scales.

P044

Expression of the hydrophobin FcHyd5p from *Fusarium culmorum* in *Pichia pastoris* and evaluation of its contribution to gushing

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Description of topic: Hydrophobins are small proteins of about 10 kDa produced by filamentous fungi with a remarkable surface activity promoting the formation of self-assembled structures known as rodlets. They are important for fungal growth and the interaction of fungi with their environment. Recent studies suggested, that hydrophobins act as factors inducing gushing for beer, the uncontrolled burst out when a beer bottle is opened. Problems of primary (biologically caused) gushing correlate with the quality of barley used in malting and occur in particular in those years when barley has been infected by fungal pathogens such as *Fusarium*.

Materials and methods for data collection: We investigated the gushing potential of the hydrophobin FcHyd5p from *F. culmorum*. FcHyd5p was expressed and secreted as a heterologous protein in an eukaryotic expression system using the yeast *Pichia pastoris*. The hydrophobin was concentrated by freeze-drying and the obtained lyophilisate applied to gushing experiments.

Results: It could be shown that amounts >0,5 mg of lyophilisate induced gushing, whereas lyophilisate of the *P. pastoris* wild type strain X33 caused no gushing. The gushing inducing property could also be observed in non-alcoholic carbonated beverages. The addition of lyophilisate to the brewing process at the beginning of mashing was also demonstrated to result in gushing beer.

Discussion: FcHyd5p, which is formed upon infection of barley by *F. culmorum*, is clearly demonstrated to induce gushing in amounts occurring in practice.

What is new - A hydrophobin was heterologously expressed in *P. pastoris*, and the purified protein was demonstrated to induce gushing.

P045

New best practise approach to traditional stabilisation

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Description of topic: Colloidal instability in beer is caused by the interaction of haze sensitive proteins and haze active polyphenols (tannoids) present in the beer. Haze formation can be controlled through the management of these haze precursors. Process aids such as PVPP and silica are employed in the brewing process to achieve the desired haze precursor levels and allow the beer to attain its predicted shelf-life.

Inefficient practises in stabilisation can result in unjustified cost waste through over stabilisation of the beer.

Implementation of Best Practise through achieving the correct dosage rate and accurate dosing can deliver optimum stabilisation while reducing the cost base to the brewery. This is a possibility with the use of the right equipment, namely the Haze Tester and the ISO-mix system.

Materials and methods for data collection: Brewery Case Study.

Results: Results demonstrate that the combination of targetted dosing and accurate, even distribution allow lower dose rates of Polyclar 10 to be employed in the stabilisation regime without compromising quality.

Discussion: Through practise of measurement of haze precursors prior to dosing of stabilisers and implementation of an accurate dosing system, significant benefits in the form of improved process efficiency are presented to the brewery. This improved process efficiency is measured through both cost and time saved.

What is new - The stabilisation regime comes under control, the brewery now has the facilities to target the correct dosing rate for the beer in question and the system of application allows for the best possible performance from the products involved in stabilisation.

P046

Gushing suppressing effects of hop constituents

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Description of topic: Gushing is one of the most unsolved problems in brewing. This phenomenon of bottled beer has been known for a long time and spontaneous foaming is caused by an imbalance of gushing promoting and preventing factors contained in beer. Despite the high diversity of gushing factors reported it is widely accepted that barley and malt contaminated by moulds represent the most significant inducing factor. They are designated as primary gushing factors and nearly all research in the last years focused on these malt and microorganism derived factors. An influence of hop is often neglected. Older reports say that hops can have an influence on the gushing tendency of beer. Beside polyphenols and bitter acids hops contain oils that give a pleasant hoppy flavour when added at late stage of wort boiling.

Materials and methods for data collection: We tested some hop derived compounds, like linalool, alpha-acids and hop oil on their gushing promoting or suppressing properties. Their effect against gushing promoted by activated carbon was investigated. More Experiments dealing with suppressing effects were carried out with a beer that showed gushing. Linalool, alpha-acids and hop oil were commercial products.

Results: These experiments showed that a concentration of 50 µg/l Linalool reduced the gushing volume by 25 %. With increasing linalool content the gushing volume decreased even more. Also hop oil significantly reduced the gushing tendency of beer. Activated carbon induced gushing which could be reduced by linalool and alpha acids. Our results were comparable with the gushing reduction obtained with a commercial hop based gushing inhibitor.

Discussion: The gushing suppressing effects of humulones and linalool were investigated and we could show that these hop constituents have an influence on gushing properties of beer. We showed that hops decreases the gushing tendency of beer when added at late stages of wort boiling. So it can be concluded that beers with higher bitter units and dry hopped beers should show a lower gushing tendency.

What is new - This is a possible explanation for the fact that some low hopped beer types are more often affected by gushing than others.

P047

Updated research results on the flavour stability of beer in plastic packagings

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Description of topic: Plastic containers and closures have taken on an important role as packaging materials for the brewing industry. Alongside the many well-known advantages there is the problem of plastic's inherent permeability to gases (O₂ and CO₂). To enhance the barrier properties of plastic material different bottle systems for example Multilayer, internal coatings and blends were developed. The quality of these various systems can be evaluated through the measurement of permeation. Since the demand of permeation measurement has reached the market novel methods for measurement are proposed and offered. The diversity of systems led to a situation in which measurement results from different sources can not be compared which each other. The lecture contains a comparison of existing methods with novel methods and at the same time an evaluation of the latest development in barrier enhanced plastic bottles and closures.

Materials and methods for data collection: The existing VLB method for the permeation measurement O₂ and CO₂ as a real time method is presented and a new method is introduced that is working with a non-destructive and non-invasive measurement. The methods are being compared.

Results: Deviations in the results of different permeation measurement methods that are not working with the real kinetic exist and must be an essential part of discussion about barrier effects.

Discussion: The existing quick permeation measurements will not substitute the real time permeation

test but may be seen as important methods to detect relevant quality deviation in delivered bottles or self-coated bottles in an incoming goods inspection and routine control for the Breweries and Bottlers.

What is new - New results of current barrier innovations on the market are evaluated.

P048

Assuring the microbiology of draught beer

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Description of topic: A number of factors influence the quality of draught beers. Notably, hygiene standards impact on the microbiological loading of beer. Elevated levels of microorganisms distort product quality and have a deleterious effect on attributes such as taste, aroma and clarity. Key to the assurance of good hygiene is regular and effective line cleaning that manages the regrowth of attached microorganisms (biofilm) within dispense tubing. We report here the implications on product microbiology of weekly line cleaning and what happens when this stops. Also a novel 'magnetic field' technology that reportedly allows the relaxation of weekly line cleaning was evaluated in terms of microbial loading in the beer.

Materials and methods for data collection: The microbial 'planktonic' loading in three draught lager beers (ABV - 3.8%, 4% and 5%) from two accounts (with throughputs of 500 and 1000 hl p.a) were monitored weekly just prior to line cleaning. Samples were taken after a litre of beer had gone to drain. Colony forming units were determined aerobically on WLD agar and anaerobically on Raka Ray (including the inhibitors cyclohexamide, vancomycin and phenylethanol) agar.

Results: Weekly line cleaning supported beers of excellent product quality with microbial loadings of 958 colony forming units (cfu)/ml (aerobes) and 665 cfu/ml (anaerobes) (average for three brands monitored weekly for 12 weeks). Stopping line cleaning for four weeks increased the average counts ca. 5-fold (aerobes) and ca.7-fold (anaerobes). The 'magnetic field' technology - on lines which were not cleaned for 9 weeks - delivered average microbial counts which were 52% (aerobes) and 32% (anaerobes) of the control beers.

Discussion: The recommended weekly line cleaning frequency is necessary to manage product hygiene. Microbial loading increases rapidly when line cleaning is stopped. A 'magnetic field' technology can play a role in relaxing line cleaning frequency (up to every five weeks) whilst maintaining low planktonic counts and without compromising product quality.

What is new - Despite its continuing and growing commercial importance, little has been published on draught beer microbiology since the early 1980's. This extended real world study updates the debate about the link between line cleaning and product hygiene. In addition, the successful evaluation of a 'magnetic field' technology is opportune as such approaches have been marketed to the on-trade for some time. This is the first time that such a technology has been evaluated in a commercial environment.

P049

Decomposition of the iso- α -acids in bottle conditioned and pasteurised beers

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Description of topic: Decomposition of iso- α -acids (IAA) was evaluated comparing bottle conditioned with pasteurised beers. In addition, the influence of the yeast strain used in the conditioning step was studied.

Materials and methods for data collection:

Samples: Five bottle-conditioned beers and a lager pasteurised beer. The conditioned beers were obtained from the lager beer (before pasteurization) using four different commercial yeast strains (one of them with two different pitching rates).

Ageing: The beers were stored for 10 months at 20°C in the dark. Analysis after 0, 1, 3, 5, 7, 10 months.

Determinations: the cis/trans IAA (by SPE and HPLC) and IBU (EBC method).

Results: The pasteurised beer was analysed before and after pasteurization. Both the IBUs and the IAA content did not change. During the refermentation step, the IAA showed an average decrease of 13%, the trans-isomers decreased more than the cis-isomers and the yeast strains did not prove different. During ageing the pasteurised beer showed a decrease in IAA by 17% and the other beers by 18% on average. The yeast strains did not prove different during ageing even if the highest pitching rate showed a strongest decrease in IAA. The T/C ratio decreased by 33% and 37% for pasteurised and conditioned beers respectively. The isochumulone, isohumulone and isoadhumulone showed a decrease during ageing by 17, 20 and 16% for pasteurised beer and 14, 18 and 19% for the other beers.

Discussion: The pasteurization did not change the IAA content even if the IAA degradation depends on temperature. During the refermentation step the IAA showed a considerable decrease, similar to what was reported in literature for the primary fermentation. The decrease in trans-isomers could be due to greater affinity for yeast by the trans IAA, but there is no data in literature in this regard. All the yeast strain tested showed the same ability to bind IAA. During ageing, the presence of the yeast in the bottle did not confer greater stability to the IAA than the pasteurised beer used as the reference.

What is new - Although the degradation of the iso- α -acids was well studied, there are still some unclear aspects. For example, the effect of the production process, like pasteurization and bottle-conditioning, on the decomposition of iso- α -acids has been poorly studied in literature. Regarding the primary fermentation and refermentation, the influence of the type of yeast strain used on iso- α -acids content, has been only recently addressed.

P050

Should brazen-equipment still be used in beer dispensing systems these days?

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Description of topic: For more than ten years different committees and boards are discussing the usage of brazen-equipment in beer dispensing systems very controversial. The repeated efforts of the "Arbeitskreis Getränkeschankanlagen des Deutschen Brauer-Bundes" to use solely equipment of stainless steel have not yet penetrated into the awareness of all persons in charge. Due to the need for scientific action investigations have been carried out to determine the release of lead from brazen-equipment which stands in contact with beer.

Materials and methods for data collection: For practical investigations the following experimental design has been used: A test rig consisting of nine beer lines (three lines at a time represent a test set-up with identical nozzles to meet statistical requirements) identical in length, diameter, construction and mountings was used. All the beer lines were equipped with four tube nozzles from brass with nickel coating. The other parts deployed were from PTFE, plastics or stainless steel to avoid additional lead release. Concerning the nozzles three different types were determined: brand-new, brand-new with damaged surface and used nozzles. The test ran for eight weeks with a weekly cleaning treatment for six hours to simulate improved aging. In addition the surface condition and the quality of galvanization of nozzles from different manufacturers were determined by the Bundesanstalt für Materialforschung (BAM).

Results: During the whole investigation period one could measure different lead concentrations released from the nozzles due to longer contact time with beer. The lead concentration appeared non-systematic and independent from age and condition of the nozzles.

Discussion: In Germany a limit value for lead in beer does not exist. In comparison to the limit value regulated by law (Germany) of lead in drinking water a repeated violation of limit was detected. The investigation results from BAM showed that the coating varied in thickness, homogeneity, adhesion

and has also scratches and flaws.

What is new - According to previous examinations on water-taps and coffee-makers now the first results can be presented for brazen-equipment used in beer dispensing systems. The lead concentrations are not hypercritical but there is a need for action. From the present results it can be derived that galvanized brazen-equipment should no longer be applied in dispensing systems and has to be substituted step by step by stainless-steel equipment.

P051

A novel method for measuring total package oxygen

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Description of topic: When it comes to product life and flavor stability of beer and beverages, oxygen remains detrimental. Preventing (even low) oxygen pick-ups during the entire production and packaging process is of paramount importance to brewers and beverage manufacturers. As a result, beer and beverages are produced with very low dissolved oxygen (DO) levels, achieving consistent quality and high flavor stability during their complete shelf life. Through a TPO measurement the brewery is able to determine the critical parameter for the sustainability of beer quality. Directly after filling, packages are prepared for TPO measurement that should be performed as quickly as possible to avoid product oxidation and consumption of oxygen. A quick determination, without sample preparation, requires separate measurement of the O₂ content in the headspace and dissolved oxygen. This results in the total package oxygen and enables to determine whether the O₂ is coming from the headspace or from the liquid.

Materials and methods for data collection: - Research

- Feasibility Study

- Pilots

- Worked together with partners

Results: A new measuring method for calculating Total Package Oxygen (TPO) out of the oxygen content in the headspace and the dissolved oxygen. The total oxygen is equal to the total oxygen calculated with the traditional z-method of Uhlig.

Discussion: A new standard for measuring Total Package Oxygen!

What is new - With the new method the total oxygen is measured fully automatically in a package that does not need to be brought to equilibrium. By measuring the O₂ content in the headspace, the dissolved oxygen and headspace volume, the Total Package Oxygen is determined. All these measurements are done without transferring the beverage out of the package.

P052

Beer categorization: A new way to understand beer expertise

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Description of topic: A beer sensory panel is a very useful tool to describe and compare beers. But training beer experts require important investments. So expertise is an important field of research in sensory analysis. Whereas effect of expertise on discrimination and description of food products has been widely studied, little is known about how sensory experts categorize their perceptions and their knowledge. By understanding how sensory experts organize their perceptions, we could develop new sensory training methods. The objective of this study was to understand how people categorize beers depending on their expertise level.

Materials and methods for data collection: Two types of beer experts were compared: sensory beer experts and brewers. We first compared their discriminative performance on blond and kriel beers. Second, the two groups of experts carried out two sorting tasks where they had to put together 1) actual beers and 2) pictures of beers they perceived similar. The sorting task on actual beers allowed us to determine whether the two groups of experts categorize beers in the same way when they taste it whereas the task performed on beer pictures allows us to determine whether the two groups of experts have a similar conceptual representation of beer categories.

Results: Globally, there is no difference in discriminative performance but it seems that discrimination criteria are not the same for the two groups of experts. We found that sensory beer experts and brewers globally categorize beers in the same way, when they taste beers or when they see beer pictures.

Discussion: Whereas categories made on beer pictures by sensory beer experts and brewers were the same as the categories that can be found in beer tasting manuals, categories made on actual beers do not correspond to the literature. So it seems that the representations of beer categories that experts have in mind are not totally based on perceptual similarities but rather on conceptual knowledge.

P053

Beer bioflavouring by refermentation: a sensorial comparison between bottle conditioned and industrial beers

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Description of topic: Sensorial comparison between bottle conditioning and isobaric bottling methods. Evaluation of the effects on the final quality product using alternative extracts for refermentation process in lager beer style.

Materials and methods for data collection: Two isobaric bottled samples, pasteurized (P) and not pasteurized (R) and six-bottled conditioned beer samples refermented with different extracts (E1, E2, E3, E4, E5, E6) have been sensory analyzed after 60 days from bottling. All beer samples came from the same non-pasteurized and matured starting batch of a bottom lager beer.

Results: Bottling conditioning can be a suitable method to obtain good quality products, even for lager beers like American lager, classic American Pilsner and German or Bohemian Pilsner beers style.

Discussion: On 30 attributes tested, 14 have shown a significant difference among samples ($\alpha \geq 0.95$). Considering the visual aspect, bottle conditioned beers show a general higher score. This could be explained by the better foam stability. Concerning flavour attributes, samples P and E4 show a higher score regarding flavour intensity, even if only last one is appreciated by panelist.

Pasteurization seems to have a negative impact on the final product but no evidence has been noticed in this study about papery flavour stale attribute. Regarding flavour pleasantness, it is possible distinguish 3 groups: the first one represented by pasteurized sample (P), which shows the worst score; the second one represented by R (isobaric and refrigerated), E2 (sucrose extract) and E6 (cane sugar extract) samples, and the third represented by E1 (treated by partial filtration and sucrose extract addition), E3 (syrup extract), E4 (craft honey extract) and E5 (industrial honey extract). Regarding taste attribute, sample E4 once again shows the best score.

What is new - The bottle refermentation could be considered an interesting tool for micro-breweries to obtain beers with a fresher aroma and taste and possibly with an improved shelf life. This can allow to avoid expensive isobaric plants, and above all, to preserve the "handmade" characteristics of the production even for lager beer styles.

P054

Occurrence of thiols in beer: Impact of bottle refermentation

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Description of topic: Thiols are known to have a strong impact on the overall aroma of fermented beverages. For fresh beers, twelve polyfunctional thiols were detected (1). All of them were absent from wort, suggesting a key role of fermentation (1). Thiols can be also generated through ageing, inducing roasted/burned (2-mercaptoethyl acetate and 3-mercaptopropyl acetate) or onion-like (2-mercapto-3-methylbutanol) off-flavours (2). In some breweries, a cold storage is applied just after refermentation to avoid milk-like defects, most probably brought by polyfunctional thiols.

As no scientific data were available on the impact of bottle refermentation on these compounds, this was the aim of the present work.

Materials and methods for data collection: An optimized extraction method procedure (3) was used to extract aroma compounds from beer. The representative extract was obtained by XAD extraction and then analyzed by GC-O, GC-MS and GC-PFPD (selective sulfur compound detection). A sensorial aroma extract dilution analysis (AEDA) was applied to determine the odor intensity of each compound (4).

Results: XAD extractions were performed on bottle refermented and unfermented beers. Some fruity esters and acids revealed detected with a higher dilution factor after refermentation. However, the biggest differences between both beers were their thiol profile. Eight polyfunctional thiols emerged as specific of the refermented beer. Their chemical structure and synthesis pathway will be discussed.

Discussion: Bottle refermentation has a significant impact on the general aroma of beer, especially its sulfur profile. Although in very low concentrations (undetectable by most usual techniques), polyfunctional thiols strongly influence the organoleptic properties of the beer due to their sub-ppt threshold values.

(1) Vermeulen et al., JAF, 2006, 54, 5061-5068; (2) Lejeune et al., Proceedings of the EBC, 2007, 836-843; (3) Lermusieau et al., JAF, 2001, 49, 3867-3874; (4) Ullrich & Grosch, J. Am. Oil Chem. Soc., 1988, 65, 1313-1317.

What is new - No scientific data were available on the impact of bottle refermentation on sulfur compounds

P055

Quantitative analysis of the content of aromatic alcohols in Czech beer using SPE and GC-MS

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Description of topic: The content of aromatic alcohols o-phenylethanol, eugenol, guaiacol, 4-ethyl and 4-vinylguaiacol, tyrosol and tryptofol considerably influence the sensoric profile of beer. As literature data concerning their content in beer in many cases differ significantly, our aim was to develop an accurate, sensitive and reproducible method for the analysis of aromatic alcohols in beer.

Materials and methods for data collection: In the newly proposed method solid phase extraction (SPE) of aromatic alcohols from beer was used. The extracted beer aromatic alcohols were subsequently quantified using GC-MS selective ion monitoring (SIM). As an appropriate internal standard, 4-ethylphenol was used.

The extraction of aromatic alcohols was performed on the 200 mg Lichrolut EN solid phase cartridge previously conditioned with 2 ml methanol and 2 ml of distilled water. GC-MS was carried out on a Trace GC Ultra gas chromatograph- DSQ II mass spectrometer system (Thermo) working in EI SIM mode. Using this method we tested various commercial Czech pale and lager bottled beer on their content of aromatic alcohols.

Results: The extraction conditions were optimized and the proposed method was validated on real samples of beer. The recovery of aromatic alcohols ranged from 78 % to 98 % and the coefficient of variation ranged from 1,22 % to 10,96 %. The limit of detection (LOD) varied from 0,001 to 0,01 mg/l.

Discussion: The newly designed method represents a simple, less time consuming, precise and accurate method for estimation of aromatic alcohols in beer.

What is new - The newly designed method represents a simple, less time consuming, precise and accurate method for estimation of aromatic alcohols in beer.

P056

Impact of organic practices on organoleptic properties of beer

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Description of topic: Consumers are more and more sensitive to the presence of pesticide residues in their products as to the negative impact of conventional agriculture on the environment. Some brewers are currently proposing organic versions of their beer to fulfill this increasing demand. However, even when an identical process is applied, organic and conventional versions of the same beer always taste differently. The aim of the present work was to point out these differences and start tracing their origin in the raw materials (hops and malt).

Materials and methods for data collection: Essential oils of organic and conventional hop samples were obtained using the adapted Likens-Nickerson method (1). An optimized XAD extraction procedure (2) was applied to both versions of the same beer. The representative extracts were analyzed by GC-MS, GC-PFPD (specific sulfur compound detection), and GC-Olfactometry. Aroma extract dilution analysis (AEDA) was used to determine the relative odor intensities (3).

Results: Differences were evidenced between the two beers. The former, already more coloured, displayed a more complex profile, with more spicy, grainy and malty aromas, whereas the conventional one contained more sulphur and stale flavours. XAD extraction was performed on both samples. Significant variations were recorded among acids, esters, volatile phenols and sulphur compounds, partially explaining tasting profile differences. Sulphur composition was tentatively linked to organic practices in hop culture, especially the use of copper sulphate.

Discussion: Beers produced with organic and conventional raw materials display very different organoleptic profiles. Most differences have been linked to hop agriculture practices. The evolution of these differences along storage is also investigated.

(1) Bouseta & Collin, JAFC, 1995, 1890-1897

(2) Lermusieu et al., JAFC, 2001, 3867-3874 ;

(3) Ullrich & Grosch, J. Am. Oil Chem. Soc., 1988, 1313-1317.

What is new - Significant flavour differences have been evidenced between organic and usual hops.

P057

Development of light beers with improved fullness and prolonged flavour stability

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Description of topic: Compared to the US, consumption of light beers in Europe is marginal. Although cultural and health-related aspects may largely explain this differential consumer behaviour, in this work we aimed at unravelling potential flavour deficiencies of light beers and to develop a fully-flavoured product with prolonged flavour stability.

Materials and methods for data collection: The first part of the work comprises detailed sensory-chemical profiling of commercial US and European light beers, in comparison with the corresponding lagers from the same breweries. In the second part, various pilot-scale brews were made in order to develop an improved process for production of full-flavoured light beers. Aiming at prolonged flavour

stability, all brews were prepared by mashing-in at 63°C and pH 5.2. Brews were made with or without addition of polyphenolic hop extract at mashing-in, in order to evaluate the influence of the reducing power during brewing on flavour stability and the impact of hop polyphenols on fullness of the finished product. Furthermore, the influence of the mash ratio, original gravity, and the use of special malts and adjuncts, was investigated. Multivariate data analysis (MVA) was performed for evaluation of all physicochemical and sensory data obtained on the fresh and aged beers.

Results: The fresh commercial beers could be differentiated based on their origin (US or European) and beer style (light or pilsner). A lack of bitterness, too much sweetness, and especially low fullness were identified as potential flavour deficiencies of fresh light beers. Upon ageing, light beers were generally less flavour stable than their pilsner counterparts, mainly due to increased sensory perception of cardboard and ribes staling flavours.

Discussion: From our own brewing trials, it was concluded that high-gravity-brewing (preferably with crystal malt; 5% of grist) and addition of hop polyphenols to the brewing liquor, resulted in light beers with improved fullness, prolonged flavour stability and higher overall sensory appreciation.

What is new - Combining extensive physicochemical and sensory analysis with MVA allowed us to identify flavour deficiencies of commercial light beers in direct comparison with their pilsner counterparts. Pilot-scale brewing trials further demonstrated that fullness and flavour stability of light beers are best served by beer preparation in the presence of high levels of polyphenols, i.e. by adding hop polyphenolic extract to the brewing liquor, combined with high-gravity brewing.

P058

Screening of different enzymes able to hydrolyse cystein adducts, a new source of hop flavours

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Description of topic: 4-Sulfanyl-4-methylpentan-2-one (4SMP) and 3-sulfanylhexanol (3SH), two Sauvignon-like flavours (1), have been detected in various lager beers and in some hop varieties (2,3,4). In wine, these polyfunctional thiols are released from S-cysteine conjugates, thanks to yeast β -lyase activity (5). The aim of the present work was to investigate how brewing enzymes could increase the hydrolysis of such conjugates, most probably present in hop.

Materials and methods for data collection: Two hop varieties were selected: Simcoe, exhibiting a sulphur-rich profile (no Bordeaux mixture treatment in the field)(3), and Nelson Sauvin, giving rise to pleasant Sauvignon-like flavours to beer. A method derived from (6) was used to quantify the S-cysteine precursors. After enzymatic digestion, hop extracts were analysed by sensorial analysis, GC-Olfactometry, GC-PFPD and GC-MS.

Results: Sensorial analyses evidenced strong differences before and after hop enzymatic digestions. GC-O, GC-PFPD and GC-MS allowed us to identify the main polyfunctional thiols derived from S-cysteine conjugates. The most efficient enzymes were selected. pH and temperature were optimized in order to increase the polyfunctional thiols recovery.

Discussion: This work highlights for the first time the presence of S-cysteine conjugates in hop. A new way to take advantage of some pleasant hop flavours is proposed.

(1) Tominaga, T.; Furrer, A.; Henry, R.; Dubourdieu, D. *Flavour Fragr. J.*; 1998; 13, 159-162.

(2) Kishimoto, T.; Morimoto, M.; Kobayashi, M.; Yako, N.; Wanikawa, A. *J. Am. Soc. Brew. Chem.*; 2008; 3(66), 192-196.

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(4) Vermeulen, C.; Lejeune, I.; Tran, T. T. H.; Collin, S. *J. Agric. Food Chem.*; 2006; 54(14); 5061-5068.

(5) Tominaga, T.; Peyrot des Gachons, C.; Dubourdieu, D. *J. Agric. Food Chem.*; 1998; 46(12); 5215-5219.

(6) Darriet, P.; Tominaga, T.; Demole, E.; Dubourdieu, D. *J. Int. Sci. Vigne Vin*; 1995; 29(4); 227-232.

P059

Identification of a new off-flavour in “light stable” beers

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Description of topic: MBT (3-methyl-2-butenethiol) is responsible of the famous lightstruck off-flavour in beer (1). Reduced hop extracts can be used to avoid iso α -acid photo-degradation (2). If it clearly improves the taste of fresh beer bottled in clear glass, some off-flavours are still detected after ageing. The aim of the present work was to investigate the pathways leading to these compounds.

Materials and methods for data collection: A commercial “light stable” beer (blend of dihydroisalpha acids), bottled in clear glass, was submitted to two conditions: natural day/night periods or dark storage. Amberlite XAD-2 resin and thiol selective pHMB extractions (3) were conducted after 0, 2, 4 and 7 months of ageing. The extracts were analysed by GC-PFPD, GC-MS and GC-Olfactometry. Expert tasting panel also detailed the organoleptic characteristics of both beers.

Results: Whereas no MBT flavour was detected by the panel, comparative sensorial analysis evidenced a strong “onion-like” off-flavour in “light stable” beer exposed to light. GC-O and GC-MS applied on the aroma extracts allowed to identify 2-sulfanyl-3-methylbutanol (2S3MB) as the key-compound (AEDA Dilution Factor = 32-1024 for 2S3MB while only 8-16 for MBT). 2S3MB revealed to be synthesized from 3-methyl-2-butenol (3MBOH) found in hop extracts. The involved radicalar mechanism is strongly enhanced by light.

Discussion: Although reduced hop extracts improve light stability regarding MBT, they give rise to strong onion-like off-flavours in presence of light : 2S3MB. The concentration of the hop allylic precursor should be controlled in hop extracts.

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P060

The sorting task: Another method to obtain beer sensory descriptions

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Description of topic: In sensory evaluation, the method commonly used to characterise and describe beers is the conventional profile. This method is very efficient but requires a lot of time to train subjects and to teach them to quantify all the relevant sensory characteristics. Recently, new faster and less restricting methods have been developed, such as free choice profiles or profile flash. In both these methods, subjects are free to choose their own vocabulary to describe beers. More recently, the sorting task (which is a method based on similarity between products) has been studied in food and non-food fields. The aim of the present study is to see whether the sorting task can be useful to obtain relevant sensory information about beers.

Materials and methods for data collection: In a sorting task, subjects are asked first to sort, into groups, beers according to their similarities. Sometimes a second step involves characterising beer groups with words. Different experimental conditions have been tested with five different sets of beers. In these experimental conditions we studied the level of training of subjects (trained and untrained subjects), the stability, the repeatability, the number of beers, the number of groups of beers to make, the use of a list of terms to help subjects to describe beers. Finally we compared the results obtained with the sorting task with those obtained with the conventional profile.

Results: Results showed that sensory maps obtained with sorting tasks are close to those obtained

with the conventional profile. Moreover, sorting tasks could be used with trained as well as untrained subjects. Using of a list of terms helped, whereas providing the number of beer groups did not. Finally, to obtain relevant results, the number of beers to classify should not be more than twenty.

Discussion: The sorting task can provide sensory maps quickly and easily. Nevertheless, as all methods where subjects are free to use their own terminology, there may be sometimes difficulties when analysing the descriptions.

What is new - Sorting task is a new method more and more used in food and non-food fields. However it has never been studied and applied to provide sensory information about beers.

P061

Relevance of non-thermal process steps on Strecker aldehyde formation after wort boiling

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Description of topic: In previous publications we pointed out the correlation between high soluble nitrogen contents in malt and wort and the increased susceptibility of the resulting beers towards beer aging. The aging process is closely linked to the Strecker degradation of the precursor amino acids. Apart from the wort boiling we elucidated further formation origins throughout the following non-thermal process steps.

Materials and methods for data collection: The studies were carried out in a 20 hl pilot plant. In contrast to the standard wort the test worts were spiked with free amino acids (FAA), namely with 125 mg/l g of methionine, leucin and phenylalanine at the beginning of boiling (BoB), at the beginning of fermentation (BoF) and into freshly bottled beer (FBB). The beers were tasted and analysed in fresh condition and after 3 and 6 months of natural aging at 28°C. The GC-MS and the amino acid detection method were described previously.

Results: In accordance to our previous results we found an increase in methional for the BoB-beer. Surprisingly this increase was also evident for the BoF-beer. This indicated that there is a non-thermal pathway to Strecker aldehydes (SA) during fermentation that is dependent on the level of the corresponding precursor amino acid. More interestingly we also saw an increased formation of SA in the BB-beer. Having an identical amount of SA in fresh condition compared to the standard the addition of amino acids intensified the amount of SA and consequently the stale flavour after 3 and 6 months significantly.

Discussion: Our results extend previous findings that emphasize on the wort boiling process as the main source of precursor generation for final SA formation in aged beer. In addition the SA formation processes during fermentation and storage must be taken into consideration. As this non-thermal pathway mainly depends on the amount of free amino acids and not only on undefined precursors generated thermally out of the FAA our request for a moderate soluble nitrogen level in the malt is confirmed considerably.

What is new - Currently the wort boiling process is mentioned as the main source of precursor generation for final Strecker aldehyde formation in aged beer. Our results clearly show that there is also a non-thermal pathway during fermentation and storage that was hardly discussed before. These non-thermal ways of formation mainly depend on the amount of free amino acids (FAA) and not only on undefined precursors generated thermally out of the FAA.

P062

Thermal influence on Strecker aldehyde formation during wort boiling

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Description of topic: In previous publications we pointed out the correlation between high soluble nitrogen contents in malt and wort and the increased susceptibility of the resulting beers towards beer aging. The aging process is closely linked to the Strecker degradation of the precursor amino acids. Currently mainly thermal processes (e.g. wort boiling) are said to be responsible for the development of Strecker aldehydes (SA). Different wort boiling times and intensities were examined to check the influences on beer aging intensity during storage.

Materials and methods for data collection: The studies were carried out in a 20 hl pilot plant. Standard worts (1-5) were boiled at low overpressure (120, 70, 60 min) and atmospheric boiling (70, 60 min). Additionally for one brew boiling was completely omitted compared to the standard wort (6+7). The beers were tasted and analysed in fresh condition and after 3 and 6 months of natural aging at 28°C. The GC-MS and the amino acid detection method were described previously.

Results: Worts 1-5 showed a slightly increased level of SA for the brew with 120 min boiling time whereas the amount of SA was equal for all fresh beers and after 3 months. Hence the tasting panel could not point out a significant difference in aging flavour for these beers. Interestingly the brew without boiling (BwB) showed a slightly higher level of SA in the fresh beer than the standard beer (SB; 70 min boiling). It became obvious that the levels of SA for BwB and SB vary significantly throughout wort production. However the final beers show a nearly identical SA amount.

Discussion: The results point out that wort boiling is not the decisive process step for determining the level of SA in final beer. Therefore measuring SA during wort production does not provide a complete picture about the SA amount and the flavour stability of the final beer. As previously published we are convinced that measuring soluble nitrogen in malt and wort is better suited to predict flavour stability and Strecker degradation of the precursor amino acids.

What is new - Published data emphasize the wort boiling process as the main source of precursor generation for final Strecker aldehyde formation in aged beer. Our results point out that wort boiling is not the decisive process step for determining the level of stale flavour Strecker aldehydes in final beer. Therefore measuring Strecker aldehydes during wort production does not provide conclusion on the Strecker aldehyde amount and the flavour stability of the final beer.

P063

Contribution of staling compounds to various types of aged flavours occurring in lager beer by studying their concentration evolutions and flavour thresholds

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Description of topic: Beer flavour is the result of a complex interaction between hundreds of compounds and even more taste and olfactory receptors. During storage, various chemical reactions occur in beer which result in the deterioration of the fresh flavour. It is however unclear which compounds are responsible for the aged flavour. Additionally, aged flavour formation (cardboard, ribes, caramel, madeira,...) appears to be dependent on storage conditions. This study gives an overview of the flavour contribution of compounds that were already linked with beer ageing and gives a clearer insight in the effect of storage conditions on the progress of ageing reactions.

Materials and methods for data collection: Thresholds were determined with the Ascending Method of Limits test as described in EBC Analytica (method 13.9). Lager beers were aged in 10 different storage conditions and the concentrations of the flavour compounds were determined with headspace solid phase micro-extraction coupled to gaschromatography-mass spectrometry.

Results: The effect of 26 staling compounds on the aged flavour of a Belgian lager beer was studied. Strikingly, thresholds were regularly found to be substantially lower than previously reported. In addition, a masking effects and various interactions were observed between flavour compounds. Finally, the progress of staling reactions was clearly dependent on storage conditions and gave rise to different staling compound concentrations and consequently different types of aged flavours.

Discussion: (E)-2-nonenal, methional, 3-methylbutanal, 2-furfuryl ethyl ether, β -damascenone and acetaldehyde were confirmed as key contributors to the aged flavour and to a lesser extent, (E,E)-2,4-

decadienal, phenylacetaldehyde, 2-methylpropanal, diacetyl and 5-hydroxymethylfurfural. Consequently, the addition of a selection of compounds could fairly well reproduce the flavour of aged lager beer. Finally, a better insight was obtained in different types of aged flavours and their underlying formation reactions.

What is new - During storage of beer, numerous reactions occur, resulting in even more flavour compounds. Therefore, studying the flavour stability of beer is very complicated and it is unclear which compounds are most important. This study gives a clear overview of the contribution of the compounds to the overall aged flavour and more specific aged flavours of pilsner beer. Consequently, it becomes apparent which compounds should be focused on when studying the flavour stability of pilsner beer.

P064

Sensory properties of iso- α -acids and their derivatives

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Description of topic: The bitterness plays an important role as hop-derived beer flavour attribute and is caused by the iso- α -acids which result from isomerisation process of the α -acids during the wort boiling process. Also tetrahydro-iso- α -acids, hexahydro-iso- α -acids and rho-iso- α -acids are widely used as post fermentation bittering products, due to their foam enhancing and light stable properties. There is only little scientific data about the sensory characteristics of these different iso- α -acid isomers and their chemically modified variants.

Materials and methods for data collection: To determine the taste properties of these bitter compounds, the isomers of the iso- α -acids and their reduced derivatives were isolated by preparative HPLC and analysed by NMR.

Results: For the sensory characterisation a panel of 12 - 20 persons (male, female) had been trained to evaluate taste thresholds and time-intensity measurements.

Discussion: These sensory data of the iso- α -acids and their derivatives were compared and discussed.

P065

LC-MS/MS studies on the influence of the pH value on the formation of novel iso- α -acid degradation products in beer

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Description of topic: The flavour of beer is heavily dependent on time of storage. The typical bitterness of fresh beer is well-known to slightly decrease in intensity and to change in quality with increasing age of the beverage. Non-volatile bitter compounds of beer have been investigated in the last decades, and it is agreed that the typical beer bitterness is caused by adding hop products during wort boiling. A number of isomerization processes during the wort boiling process have been reported to be of major importance for bitter taste development in the final beer product. Moreover, the iso- α -acids have been identified as the major bitter contributors in beer and were demonstrated to be generated upon a re-arrangement reaction of their hop-derived precursors, namely the α -acids. Already De Cooman et al. 2000 pointed out that particularly the trans-iso- α -acids are prone to degradation. In contradiction to previous findings, Intelmann and Hofmann revealed an acid-catalytic

decomposition pathway for trans-iso- α -acids to tri- and tetra-cyclic degradation products. In the present study, we investigated the influence of the pH value of beer on the formation of these degradation products by means of quantitative HPLC-MS/MS experiments.

Materials and methods for data collection: Beer was adjusted to various pH values and the profiles of iso- α -acids and their corresponding degradation products in fresh and aged beer were monitored by quantitative LC-MS/MS analysis using the multiple reaction monitoring (MRM) mode.

Results: The results exhibited a negative correlation between the pH value, the instability of iso- α -acids, and the formation of their tri- and tetra-cyclic degradation products.

Discussion: The newly discovered degradation products were demonstrated to be the major beer aging indicators explaining almost all of the loss of trans-iso- α -acids in aged beer samples. Due to the new findings, the brewing process is to reconsider with respect to pH modification since already a slight raise of pH shows an effect of flavour stability of aged beer.

What is new - This investigation clearly demonstrates that, besides aroma-active volatiles, also the generation of nonvolatile degradation products originating from bitter-tasting iso- α -acids contribute to the flavour instability of beer and underlines the complex nature of the deterioration of beer products. The industrial importance of this new finding was confirmed by a quantitative monitoring of the novel degradation products in real beer samples taken from a brewery production line.

P066

Interactions in beer flavour release and perception

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Description of topic: Much is known about the role of individual ingredients and of key process parameters in determining beer flavour quality. However, relatively little is known about the sensory and physicochemical interactions between key flavour components - for example the impact of carbonation on perceived bitterness, sweetness or aroma of beers. The aim of this investigation was to evaluate interactions between selected primary flavour determinants in beer (carbonation level, alcohol content, bitterness).

Materials and methods for data collection: Model systems are frequently used in scientific experiments when independent control of attributes is required in order to decouple the effects of different factors. This would be impossible in a brewed beer. For this investigation a model beer system with a typical lager flavour was blended from representative ingredients. A full factorial experiment was conducted where the sample composition varied in terms of hop acid content, ethanol content and carbonation level. Aroma release from these samples was measured in vivo using Atmospheric Pressure Chemical Ionisation Mass Spectrometry (APCI-MS).

Results: Ethanol significantly increases the release of ethyl acetate, phenethyl alcohol and isoamyl alcohol ($p < 0.05$). Carbonation significantly increases the release of ethyl acetate and isoamyl alcohol ($p < 0.05$). Hop acids did not affect volatile release.

Discussion: Results suggest the key area of release in vivo is at the surface as opposed to the bulk liquid. Results depended on partition coefficients of volatiles, surface generation (carbonation) and interaction with surface active compounds such as ethanol.

What is new - The effects of ethanol (<5%) and carbonation on volatile release in-vivo using a model beer system.

P067

Determination of flavour active monophenols in beer using liquid-liquid extraction with pH adjustment and GC-MS

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Description of topic: The volatile phenols are a group of flavour compounds that have been extensively studied in wine and other alcoholic beverages, where they are often held responsible for certain vanilla like, spicy and woody aromas. In beer however, the research on phenolic compounds was mostly focussed on phenolic acids and anti-oxidants and not so much on the flavour active monophenols. Therefore, a method was developed to investigate these simple phenols in beer.

Materials and methods for data collection: A triple liquid-liquid extraction method with pH adjustment, using CHCl₃: MeOH (3:1 v/v), KOH-solution (10% w/v) and CH₂Cl₂ as extraction solvents, was improved to isolate monophenols from beer samples. Beer was spiked with 16 reference compounds and subjected to several extraction conditions. The obtained extracts were analysed by direct injection GC/MS, using a column with mid-polar stationary phase. Commercial beer samples were analysed with the optimised method.

Results: After extraction with KOH-solution the pH was adjusted to different values, ranging from pH 9 to pH 6. This influenced the amount of extracted phenols, dependent on the functional groups present. Various monophenols could be identified when analysing different commercial beer samples with the improved method, including vanillin, acetovanillon, methyl vanillate, 4-hydroxybenzaldehyde, thymol and syringaldehyde. Many of the identified phenols haven't been reported as beer compounds previously.

Discussion: The selectivity of the optimised extraction method is based on the typical acidity of the hydroxyl group of these phenols. With a pKa between 8 and 10, phenols are weak acids, appearing in their molecular form when the pH lies well below their pKa value. The difference in extraction efficiency for various substituted phenols could be explained by their slightly varying pKa values. In various Belgian beer styles flavour active monophenols could be detected, which proves that the optimised extraction method is suitable for the identification of these phenols in beer samples.

What is new - Flavour research on beer with a specific focus on monophenols hasn't been performed before, despite their promising flavour profiles. They are usually not detected using standard methods, due to their low concentrations, reduced volatility and mid-polar nature. Therefore, goal-oriented research is necessary to investigate the presence of these monophenols in beer and their influence on the beer flavour, which might be important for control of the flavour balance and for product diversification.

P068

Identification of novel unique flavor compounds derived from Nelson Sauvignon hop and synergy of these compounds

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Description of topic: Nelson Sauvignon(NS) is a unique hop variety that was bred and grown in New Zealand. This hop belongs to the high alpha-type hop, but it gives a specific floral flavor like Sauvignon Blanc wine to finished beers. We have already identified isobutyric esters and a new volatile thiol, 3-sulfanyl-4-methylpentan-1-ol, derived from NS. This result has been reported at EBC 2007. Now, we studied how these compounds contribute to the characteristic flavor of the beer made from NS (NS Product).

Materials and methods for data collection: All the hop varieties that we used were commercial ones. Test-beers were brewed in pilot -scale brewing apparatus. The volatile thiols were specifically extracted by reversible combination of the thiols with sodium p-hydroxymercuribenzoate (p-HMB) as described by Tominaga et al. These volatile thiols were analyzed by using GC-FPD, GC-O and GC-MS. Sensory evaluation was performed by 10-13 well-trained panelists.

Results: We have already reported that isobutyric esters (isobutyl isobutyrate (IBIB), isoamyl isobutyrate (IAIB) and 2-methylbutyl isobutyrate (2MIB)) and a new volatile thiol, 3-sulfanyl-4-

methylpentan-1-ol (3S4MP) were found in the NS hop and the NS product. Isobutyric esters had a floral flavor like green apple and/or apricot. 3S4MP had a grapefruit-like and/or rhubarb-like odor, similar to that of Sauvignon Blanc. Now, we newly identified another volatile thiol, 3-sulfanyl-4-methylpentyl acetate (3S4MPA), in the NS product. This compound had a grapefruit-like odor, similar to that of 3S4MP.

Discussion: We quantified these compounds in the NS products and determined their thresholds. As a result, 3S4MP was contained threefold of its threshold in beers and 3S4MPA and 2MIB were contained below their thresholds. However, it was confirmed that 3S4MP enhanced the flavors of 3S4MPA and 2MIB by synergy. Therefore, we concluded that all of these compounds would contribute to the specific odor of beers produced with NS.

What is new - To our knowledge, none of these two volatile thiols, 3S4MP and 3S4MPA, had ever been described in wines and other foods before. And we confirmed that 3S4MP enhanced the flavors of 3S4MPA and 2MIB by synergy.

Our company released the commercial beer product using NS hop in Japan. This product has won Best in Class (Class: Experimental beer) at 2008 New Zealand International Beer Awards.

P069

Masking solution for off-flavours In alcoholic beverages: The Olfactoscan®

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Description of topic: The formation and presence of volatile off-flavours in foods and beverages is a widespread and costly problem for the food and beverage industry. If the removal of an off-flavour is not feasible other volatiles can be used that mask or suppress the perception of the off-flavour [1,2]. The concept of off-flavour masking is of great interest for the food and beverage industry.

Fermentation and hop selection in combination with brewing optimization could lead to a considerable product improvement once knowing which aroma-aroma or aroma-taste interaction have masking or enhancing properties [3]. However, predicting which odorants mask or suppress the perception of a selected off-flavour is difficult and often the result of time-consuming trial-and-error methods. In this poster examples are given for the masking of potato-like off-flavours in alcoholic beverages with the help of an unique in-vivo screening technique called the Olfactoscan®. The Olfactoscan® allows a rapid sensorial determination of masking and/or suppressing odour interactions (4). Besides aroma-aroma interaction also aroma-taste interaction can be studied. As this method is based on the sensorial evaluation of odour interactions, it includes masking as well as suppressing effects at all levels, namely at receptors, olfactory bulb as well as olfactory cortex.

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Materials and methods for data collection: x

Results: x

Discussion: x

P070

Improving flavour stability of the final beer through the use of innovative natural antioxidant

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Description of topic: Flavour stability is very significant to evaluate beer quality. The beer occurs during the all production many problems of oxidation. Glutathione (GSH) is one of the most abundant low-molecular-weight thiols found in living cells. This study presents industrial results about the use of a natural and innovative yeast extract with high level content of GSH. The objective of this study is to stabilise the organoleptic profile of the final beer by inhibiting the oxidation of the lipids, proteins and reductones in further aldehydes and free radicals compounds leading to the stealing agents, introducing this selected yeast extract peptides such as GSH during maturation, in order to bring a new alternative in regards to the latest legislation on antioxidants.

Through a limit of 10 ppm of sulphites in the final product, the brewers start to reduce the oxidation acting directly on precursors from the brewhouse (Lox). However it remains necessary to insure a protection during bottling.

Materials and methods for data collection: HPLC-UV/GC-MS/SNIFFING/SPECTRO

Results: A large panel of treatments and analysis from several pasteurised lager and speciality beers has been carried, through a comparative test between traditional antioxidants and the innovative yeast extract GSH, from the maturation tank or the bottling line. The beer shelf life from 9 to 15 months is quantified by measuring ITT and RSV-TBARS. Aldehydes, sulphur compounds, amino acids, fatty acids are quantified by GC-MS. More traditional analysis are mentioned, such as the effect of the turbidity, colour, consequence on foam, impact on protein and polyphenols, heavy metals and total sulphites before and after treatment.

Discussion: A production scheme illustrates the major steps of selecting the antioxidant fraction from a yeast extract *S. Cerevisiae*. A panel of beer specialists has evaluated the beer ageing through triangular tests: such alternative would be recommended as an essential action to preserve or increase naturally the quality and standardization of the final beer.

What is new - This new technology based mainly on the GSH antioxidative potentiality allows the brewers to an optimal antioxidation during the bottling increasing at the same time the shelf - live of the final product and complaining to the legislation.

P071

Laboratory- and pilot plant-scale study on the creation of carbonyl compound-sulphur dioxide adducts

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Description of topic: Delaying flavour staling, in order to prolong shelf life of beer, is one of greatest challenges that the brewing industry is facing today. While the influence of free carbonyl compounds on beer quality is more or less solved, the role of carbonyls bound in adducts with sulphur dioxide formed during the fermentation process is still quite underestimated. Reverse degradation of these compounds can totally change the flavour of beer, especially after long storage under improper storage conditions.

Materials and methods for data collection: Model solutions of carbonyl compound-sulphur dioxide adducts were prepared by mixing of standard solution of sodium sulfite with various amounts of selected aldehydes. The content of free and bound sulphur dioxide was measured by flow-through chonopotentiometry method in this model solutions as well as in worts and green beers made under pilot-scale conditions.

Results: Intensity of the creation of adducts is basically pH independent under common conditions of the brewing technology (pH 4-7). The critical parameter for stability of adducts is temperature. Temperature increase from 0°C to 50°C results in 50% decrease of the adducts content. The next relevant parameter is the structure of applied carbonyls. As expected, short linear carbonyl compounds provided higher yield ratio than the other carbonyls. The reaction yield was also affected by molar ratio of reactants. From the technological point of view, the critical part of technogy for creation of adducts was secondary fermentation, during which adducts content increases more than twice.

Discussion: Hypothesis that the creation of carbonyls-sulphur dioxide adducts under conditions of the fermentation is possible was proved. The most important factors that affect this reaction are temperature, the structure of carbonyl compound and molar ratio of reactants. The critical technological steps for this reaction are end of the main fermentation and the maturation of beer.

What is new - Carbonyl compounds, masked in adduct form by reaction with sulphur dioxide during fermentation step, are transferred into the final product. Under certain conditions, these masked carbonyls may become the source of undesirable sensoric changes, even if conventional analytical methods show no relevant reason for such changes.

P072

Quantification of flavour active cis- and trans-(4,5)-epoxy-2E-decenals in beer by GC-NCI MS with isotope standards

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Description of topic: cis- and trans-4,5-epoxy-2E-decenals are highly flavour active compounds. They are resulting from the peroxidation of linolenic acid. Epoxydecenals are very reactive compounds; therefore, their accurate analysis requires isotope standards.

Materials and methods for data collection: Deuterated and C-13 labelled reference standards of cis- and trans-4,5-epoxy-2E-decenals were successfully performed. Synthesis: Carbon chain elongation with C-13 labeled potassium-cyanide of 1-bromo-3-nonyne and saponification of the nitrile gave methyl 1-13C-4-decyneate. The triple bond was reduced to the cis double bond with deuterium gas and the ester group is reduced to the aldehyde. Epoxydation of the double bond and desaturation at C2-C3 yielded 1-13C-4,5-2H2-Z-4,5-epoxy-2E-decenal. The trans isomer was synthesised by a different strategy. Beer sample work up was performed with Kutscher-Steudel extraction and "SAFE" sample preparation. Analysis was performed by GC-MS in the negative chemical ionization (NCI) and SIM mode.

Results: A simple way for the synthesis of high reactive cis- and trans-4,5-epoxy-2E-decenals was used to achieve isotope standards. Beer work up procedure was optimized and GC-MS Analysis with NCI mode enabled limits of detection for epoxydecenals with 10 ng/l (0.01 ppb) in beer. The content of Epoxydecenals in beer (fresh) varied from 0.02 ppb (fresh beer) to 0.4 ppb (40 °C, 3 days). The flavour of the cis-epoxy-2E-decenal was confirmed to be metallic, while the trans-epoxide was classified as malt-like.

Discussion: The new synthesis method allows the easy introduction of isotopic labels in nearly all positions of the epoxydecenals and therefore an isotope dilution assay was successful. Optimized sample work up, especially the usage of solvent assisted flavour evaporation (SAFE) and NCI-MS enabled the quantification of the target epoxydecenals at ultra trace amounts (ppt).

What is new - First time analysis of flavor active epoxydecenals in ultra trace amounts in beer and demonstration of its formation / release during aging.

P073

Comprehensive chemical-analytical profiling and characterisation of Belgian 'kriek' beers

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Description of topic: Fruit beers, appreciated worldwide, represent a small but rising segment in the

Belgian beer export, amounting to a volume which should not be underestimated. The fruit beer 'kriek' is in origin made by addition of fresh sour cherries to mid-young lambic beer. The traditional kriek beer is less fruity and more acid. As fresh sour cherries are limited available, and due to a trend towards sweeter beers, commercial kriek beers are nowadays often sweetened and prepared by use of cherry juice and aroma, which can be entirely or partly substituted by colourings and flavourings, resulting in a wide variety of beers. Forced by the international trade and increasing competition with other beers, this study is the first to aim at a detailed characterisation of a number of kriek beers to bear mutual comparison or comparison with other beverages.

Materials and methods for data collection: The characterisation of the Belgian kriek beers is performed by analytical flavour profiling, including higher alcohols, ethyl esters, acetate esters and aldehydes, by headspace solid phase microextraction in combination with gas chromatography-mass spectrometry (HS-SPME-GC-MS), and by quantitative determination of the bittering profiles, organic acids and sugars by high pressure liquid chromatography.

Results: An analytical HS-SPME-GC-MS method has been developed for reproducible flavour profiling. In the complex volatile fraction of all beers, 103 compounds were identified. The selected marker aldehydes, ethyl and acetate esters, higher alcohols, organic acids and sugar profiles varied between all beers. Many hop degradation products were noticed due to the use of aged hops. After 1 year of spontaneous ageing, minimal deterioration is observed.

Discussion: Based on the resulting data matrix, great diversification is noticed between the commercial kriek beers investigated in this study. A clear classification of the beers, resulting in brand recognition, is obtained by application of principal component analysis (PCA) on the complex analytical data matrix.

What is new - This comparative study is the first to examine the chemical-analytical differences between spontaneous refermented (traditional) kriek beers on the one hand and sweetened kriek beers on the other hand. The origin of the lambic beers used for kriek beer production is completely unscrambled. The link between the kriek juice extract and the final aroma profile of the fresh kriek beer is revealed. Flavour profiling based on HS-SPME is considered a powerful tool underpinning these recent findings.

P074

The effect of CO₂ extracted malt on flavour and foam stability

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Description of topic: CO₂ can be used as a solvent for the extraction of mainly non polar substances with moderate thermal stress for the raw material. Consequently pale malt was extracted with supercritical CO₂. Fatty acids like palmitic acid, oleic acid, linolic acid and linolenic acid act as a source for ageing active aroma compounds and foam destroying substances in beer. By decreasing those substances with supercritical CO₂ as a solvent, positive effects on taste and foam stability occurred.

Materials and methods for data collection: Fatty acids were analysed by High Performance Gas Chromatography. The basic wort analyses like extract, alcohol content and pH-value were executed due to Analytica EBC and MEBAK. Foam analyses were done by NIBEM foam-tester. The fresh and forced aged beers were tasted according to the DLG and the EICHHORN scheme.

Results: The trials have been executed with different additions of defatted malt. By using pure defatted malt for the brewing trials an improvement of foam and an increase of taste stability could be achieved. Furthermore the augmentation of aging indicating substances in beer like 3-methyl-butanal, 2-acetylfuran or gamma-nonolacton was considerably less compared to the trials with standard malt. Hence not only analytical but also sensory analyses indicated a better beer quality.

Discussion: It is well reputed that fatty acids work as precursors for substances that degrade taste stability when reacting with oxygen. Up to now common methods to diminish those reactions have been the reduction of oxygen during the brewing process. By using CO₂ for the extraction oxygen could be partly displaced when CO₂ gasses out during the mashing process. But the main benefit of the extraction was the reduction of fatty acids.

What is new - A new approach is to reduce the fatty acids in the corn by an extraction with supercritical CO₂. The method provides defatted malt with comparable enzymatic power to non defatted malt and a protection against oxidation during the mashing process.

P075

Decision maker tool for evaluation of T2 and HT2 toxins contaminated malting barley

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Description of topic: The aim of this study is to better characterise consequences of barley kernel infection by *Fusarium langsethiae* in order to help maltsters from using TCT A highly contaminated barley samples. We have acquired data concerning the infection symptoms on barley ear by using molecular tools and tried to determine best method for TCT A highly contaminated grains elimination.

Materials and methods for data collection: Barley samples issued from experimental trial were processed as close as possible to common malting practices. *Fusarium* spp. and *Fusarium langsethiae* detection and quantification were realised by using specific primers and a real-time PCR quantification. In the same time, trichothecenes were assessed by HPLC MS/MS.

Results: Before malting, barley kernel calibration drives to the elimination of small grains. The analysis of 4 calibration subsamples shows that the smallest grains sample displays a very high amount of T2 and HT2 toxins correlated with a high content of *Fusarium langsethiae* DNA, whereas the other fractions are less contaminated. In addition, barley ears displaying various symptoms were analysed in the same way by real time PCR. The DNA amount of *Fusarium* spp. and *Fusarium langsethiae* was determined for each grain of these ears. This study revealed that *Fusarium langsethiae* infection can be associated with apparition of small and fit black (or not) grains.

Discussion: During harvest and calibration before malting, most of small grains are eliminated. Thus a major part of highly contaminated grains would not enter the malting process. Then barley calibration before malting is the best way to prevent contaminated malt.

What is new - Symptoms linked to *Fusarium langsethiae* infection are not actually known. In this study real time PCR is used to quantify *Fusarium langsethiae* in the total *Fusarium* spp. on each single kernel of a spike. Therefore, this method allows to realise spike cartography and to associate *Fusarium langsethiae* to infection symptoms. This can lead to early detection of high risks parcels and to prevention of highly contaminated barley samples.

P076

Rapid detection of yeast in brewery rinse water

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Description of topic: Vessels utilized within the brewing industry are sterilized or sanitized after use to prevent contamination from unwanted particulate matter, chemicals or microbes. While the efficiency of cleaning agents is typically good, it is common practice to perform tests on rinse water to ensure that hygiene standards are met. Recently there has been a growing trend towards the implementation of quick and reliable PCR-based methods for the detection/identification of bacteria or wild yeast contaminants in brewery samples. However, in many instances pre-enrichment for 16-72h is required prior to analysis and the level of differentiation provided is excessive for basic hygiene assessment. Here we describe a simple method to detect yeast as a means of assessing vessel hygiene.

Materials and methods for data collection: In this study we demonstrate the application of a novel hollow fiber filtration module (Elutrasep™ device) to filter yeast cells from a large sample volume, while allowing them to be eluted in smaller quantities (<1ml). Brewery rinse water samples were filtered and analyzed using Q-PCR (Lightcycler and TaqMan systems) in conjunction with primers designed to be specific for the *Saccharomyces* genus in order to quantifiably detect yeast.

Results: Elutrasep filtration allowed a similar number of cells to be recovered to standard membrane filtration (>80%). However the ability to recover cells to be used directly for Q-PCR analysis resulted in an extremely low detection limit of approximately 1 cell/2ml of brewery rinse water, irrespective of the Q-PCR system employed. Further analysis of brewery samples using Q-PCR revealed that yeast could be reliably detected at this level while no false positives or negatives were observed.

Discussion: The method described may be used to detect yeast in rinse water samples in <5 hours. Consequently, a rapid assessment of microbial loading can be performed, aiding the implementation of effective HACCP monitoring and allowing proactive decisions to be taken regarding vessel hygiene.

What is new - As a means of obtaining cells for use in PCR, the novel Elutrasep device displays significant benefits over other recovery methods. In addition, recovery of cells in this fashion circumvents the need to pre-enrich samples, leading to a faster response time. Here we report the first results where Q-PCR is used to detect *Saccharomyces* yeast as a QC tool for vessel hygiene.

P077

Moulds presence management for a better Gushing risk control

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Description of topic: For several years, the mould presence on grains after storage and its impact on quality have been studied. An Innovant Tool has been developed to manage the presence of moulds on cereals. For brewing, the topic is to focus on the moulds which can provide Hydrophobins. Those Hydrophobins are small fungal proteins isolated in *Fusarium*, *Aspergillus*, *Nigrospora* and other isolated strains.

Materials and methods for data collection: The new tool is based on the measurement of the Activity of water and the temperature during the barley storage. The Thermodynamical parameters of the product are managed to have the barley maintained in safe conditions. The control of the Activity of water avoids the fungal development (*Aspergillii* and *Penicillia*). It also has the role of destroying and/or enactiving the existing fungal population (*Fusaria*). The Activity of water and the temperature sensors are installed in the cereals storage. It needs in situ measurement equipment.

Results: This approach of the storage decreases the quantity of mould present on the grains after the storage period. Consequently, it also reduces the Hydrophobin presence. Based on correlations observed between the Hydrophobin level and the gushing potential of barley and malt, the result is a better gushing risk management.

Discussion: This tool has been created for the brewers who have understood that the fungal Hydrophobin has an important role in the Gushing activity of barley and malt. This Innovative mould presence management has other effects. For example, it is a good tool for Mycotoxin control. The user cost is between 0.5 and 1 € per ton. It is enough low to be applied in the agroindustry.

What is new - The innovative approaches are based on a [Aw, T, t] in situ management associated with a better knowledge of the [Aw, mould reaction].

P078

Anti-bacterial activity of lysozyme in pitching yeast and effect of lysozyme on yeast fermentation

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Description of topic: Lysozyme has anti-bacterial activity against Gram-positive bacteria and has no activity against yeast. As such, lysozyme can be used for the specific inhibition of beer spoilage bacteria. After all, the most frequently identified beer spoilage bacteria are Gram-positive lactic acid bacteria, and brewers' yeast is the culture used for brewery fermentations. Hen egg white lysozyme (300mg/L) is tested for the antibacterial activity against four described lactic acid bacteria in industrial pitching yeast and in industrial beer with refermentation in the bottle. The four studied lactic acid bacteria strains are sensitive to the antibacterial effect of lysozyme. The sensitivity is species dependent and probably influenced by the characteristics of the yeast slurry. The influence of industrial pitching yeast treated with lysozyme on the fermentation performance is also studied. No negative effect is observed on the yeast fermentation performance in any of the tests carried out.

Materials and methods for data collection: Part I; Antibacterial activity of lysozyme in pitching yeast
Part II; Effect of lysozyme on yeast fermentation
Part II; Antibacterial activity of lysozyme in refermented beer and effect of lysozyme on yeast fermentation.

Results: Part I; The four studied lactic acid bacteria strains are sensitive to the antibacterial effect of lysozyme. The sensitivity is more pronounced when the yeast suspension is incubated at 22°C.

Part II; After one day incubation of yeast slurry with lysozyme at 22°C, no negative effect of lysozyme addition on the fermentation performance was observed.

Part II; The lysozyme had no negative effect on the refermentation properties of the yeast. The four studied lactic acid bacteria strains are sensitive to the antibacterial effect of lysozyme.

Discussion: The antibacterial activity of lysozyme is clearly most effective for inhibiting growth of bacterial cells.

What is new - a). Further research on effect of lysozyme to prevent or delay growth of beer spoilage bacteria

b.) Effect of lysozyme on the microbiological stability and sensory attributes of beer

c.) Identification of beer spoilage bacteria in samples of unpasteurized beer, pitching brewing yeast and wort.

P079

A new method to determine yeast viability by phase contrast microscopy

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Description of topic: A yeast viability method based on phase contrast microscopy without the use of dyes is reported, with the aim to develop a fast, simple and accurate methodology to be used in a routine basis in every alcoholic fermentation processes.

Materials and methods for data collection: Five yeast strains were grown on 100.0 mL of sterile YEPD media (16h/28°C) after that cells were inoculated in sterile mash. The yeast cells were incubated at 33°C/24 h. Initially two samples of each strain were prepared. The first sample from a fresh culture containing a population of cells with 100% of viability. The second sample was prepared from the same population, except that the yeast received a treatment with 10% ethanol and was heated at 65°C for 20 minutes to kill all cells. Different proportions of the two samples (alive and dead) were combined in order to get a yeast cells populations ranging from 0 to 100% viability. The yeast viability using the phase contrast method was compared with the erythrosin B and methylene blue (Standard methods).

Results: Phase-contrast microscopy analysis revealed that the living cells presented dense and dark, and dead cells were shown to be bright, oval and slightly smaller than living cells. The combination of these characteristics made easier the identification of cells that were actually dead in the studied populations. This represents an advantage compared to the staining methods and fluorimetry to determine the viability.

Discussion: Yeast viability can be fast and easily determined with precision and accuracy by phase-contrast microscopy, since this methodology showed excellent correlation with the standard methods, does not require dyes and buffer solutions as occurs when using colorimetric methods, and it is not subject to nonspecific reactions caused by photoreactivity and still has an advantage over fluorescent methods because require simplest accessories and lower cost that can be easily installed at any time in conventional optical microscopes widely used in the distilleries and breweries worldwide.

What is new - It is a new method to determine the yeast viability in fermentation processes.

P080

Culture-independent PCR-DHPLC technique for profiling of microbial communities in malting and brewing process

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Description of topic: Complex microbial communities are an integral part of cereal ecosystems. Microbes greatly influence performance of the processes and the safety, technological, nutritional and organoleptic properties of the final products. Therefore, understanding and routine monitoring of microbial population dynamics in the processing is important. In our previous study, the polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) was shown to be a useful tool to explore dynamics of predominant bacterial populations in the malting ecosystem. Moreover it revealed previously unidentified species. However, this technique is rather time-consuming and therefore not optimal for analysis of large amounts of samples. The present study was undertaken to evaluate the potential of automated denaturing high performance liquid chromatography (DHPLC) for microbial community profiling which allows PCR-amplified fragments with different sequence to be separated without a gel.

Materials and methods for data collection: Genomic DNA was extracted from pure cultures and real samples using FastDNA SpinKit for Soil (Q-Biogene). The target sequences were amplified by PCR and separated by DHPLC using WAVE Microbial Analysis System connected to fragment collector (Transgenomics).

Results: DHPLC allowed separation of the polymorphic PCR-amplified fragments from reference strains and their mixtures. The analysis of cereal samples at various process stages showed that the technique has potential to study microbial diversity and dynamics in the malting and brewing. Fraction collection and DNA sequencing from well-separated profile peaks allowed identification.

Discussion: DHPLC was found to have benefits over DGGE analysis, including speed, automation, convenience and reproducibility of the fingerprints. It enabled the production of community fingerprints of individual samples within minutes. The fragment collection facilitated identification of resolved peaks. DHPLC has a wide range of possible applications in the beer production chain.

What is new - PCR-DHPLC was applied for the first time to study microbial diversity and dynamics in the malting and brewing process.

P081

The survival of pathogens in wort and beer - the ethanol and pH hurdles

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Description of topic: Beer is typically free from pathogenic microorganisms due to combined effects of a series of antimicrobial hurdles. These inhibitory factors include the presence of a low pH, ethanol,

hop iso- α -acids and carbon dioxide, and reduced levels of oxygen and available nutrients. However, beers with reduced levels of these hurdles are more susceptible to the growth and/or survival of pathogenic bacteria. In this study we show the effect of ethanol and pH on the survival of four foodborne pathogens (*Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella Typhimurium* and *Staphylococcus aureus*) in wort and beer.

Materials and methods for data collection: Overnight microbial cultures were prepared and inoculated into triplicate flasks of wort or beer with various levels of the inhibitory factors under investigation. Enumeration was performed on nutrient agar using standard spread plate techniques.

Results: No growth was observed at pH values below 4.0, or at ethanol levels above 4%. However, growth and survival were enhanced as pH levels were raised and ethanol levels were lowered in both the worts and beers.

Discussion: Growth and survival of all four pathogenic bacteria under investigation was minimal at "normal" beer pH and ethanol levels. However, when the levels of these two antimicrobial hurdles were reduced, growth was supported and survival was increased. Therefore, special attention should be paid to beer products with reduced levels of intrinsic protection.

What is new - In this work we show the contribution that ethanol and pH make to protect beers from the growth of *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella Typhimurium* and *Staphylococcus aureus*. We observed enhanced growth and survival as hurdles were removed.

P082

The antifungal protein from *Aspergillus giganteus* fights filamentous fungi on barley

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Description of topic: Contamination and destruction of crop, plants and biomaterials by filamentous fungi is responsible for enormous economic losses worldwide. Head blight caused by *Fusarium* species is mainly accountable for crop loss throughout the world. However, crop loss is not the only threat; also mycotoxin formation and fungus specific metabolites reduce barley based product quality. The filamentous fungus *Aspergillus giganteus* produces a selective antifungal protein that has fungicidal properties at a micro molar concentration. It has been demonstrated that the *Fusarium* species, in particular, is very sensitive towards the so called "Antifungal Protein" (AFP). Remarkably, the growth of bacteria, yeasts, plant- and human cells is not affected.

Materials and methods for data collection: We have been testing AFP application during the malting process using natural contaminated barley.

Results: First results indicate that indeed AFP application during malting is able to inhibit the growth of *Fusarium* species on barley, whereas malt quality was not affected. Current investigations are focusing on the course of mycotoxin concentration, gushing potential of the treated malt and influence of AFP on the end product beer.

Discussion: Current results are very encouraging, indicating that AFP could be used during the malting process of barley to minimize or even annihilate the risk of mycotoxin formation and furthermore the production of fungal metabolites (gushing factors) that reduce the quality of malt based beverages.

P083

Polymer pipelines for beer and softdrink - an economic alternative to stainless steel

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Description of topic: In 2006 Carlsberg evaluated economic alternatives to stainless steel pipelines for transport of beer. Two different pipe systems have since been developed and qualified, which were not commercially available. In 2008 continuous polymer pipelines have been installed at the Carlsberg brewery in Fredericia (Denmark) to connect the brewery with the neighbouring filling lines at a distance of 1.200 m.

Materials and methods for data collection: This paper describes the requirements for polymer pipelines in the beer and food industry. The organoleptic properties of the polymer pipe system are most important. Tests according to Regulation (EC) 1935/2004 as well as tasting tests have successfully been performed. Another important requirement is for the pipes to withstand "cleaning-in-place" (CIP) conditions at high temperature with caustic. This requires good chemical and thermal resistance of the polymer as well as low thermal conductivity. In addition the pipes have to have a low permeability to carbon dioxide and oxygen to maintain the quality of the beer.

Results: Continuous polymer pipelines have been qualified which are very economic compared to stainless steel pipes.

Discussion: --

What is new - The installed continuous pipelines are the first of their kind used in the brewing and food industry for process purpose.

P084

Fluxcalcination of Kieselguhr - influence of the fluxing agent on formation of crystalline phases, permeability, colour and suitability as filter aid

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Description of topic: Kieselguhr is a sedimentary rock mainly composed of opallike, amorphous silica, which is worldwide used for deep bed filtration processes. Prior to using kieselguhr as a filter aid, the raw material has to undergo a conditioning process. Fluxcalcined kieselguhr is manufactured by treating the kieselguhr in a kiln at 900°C - 1200°C after adding an alkaline flux, generally sodium carbonate. Most of the amorphous SiO₂ is transformed into a crystalline phase of SiO₂ called cristobalite which can cause silicosis and is suspected to cause cancer. Therefore the influence of fluxing agents on the formation of cristobalite during the fluxcalcination of kieselguhr was investigated.

Materials and methods for data collection: Dried kieselguhr was treated in a muffle kiln and a rotary kiln, after adding different fluxing agents. Amorphous and crystalline SiO₂ phases were differentiated via X-ray diffractometry.

Results: Kieselguhr fluxcalcinated with fluxing agents containing sodium showed the tendency to form crystalline material. Using these fluxing agents the highest permeability and best colour values could be achieved. Fluxing agents containing potassium ions showed a significant lower tendency to form crystalline material. Permeability was lower compared to fluxing agents containing sodium. Colour measurement showed considerable red values.

Fluxing agents containing magnesium or calcium did almost suppress formation of crystalline phases. On the other hand permeabilities of these samples were significantly lower. Colour values were also insufficient.

Discussion: Fluxing agents containing potassium ions are suitable to lower the formation of crystalline material during fluxcalcination of kieselguhr. Samples fluxcalcined in the rotary kiln showed acceptable permeabilities and were suitable as coarse kieselguhr for filtration of beer. Filtration results showed almost equal turbidity of beer compared to using conventionally fluxcalcined kieselguhr as coarse kieselguhr. Merely pressure difference was higher.

What is new - The novel approach of the research project is the use of alternative fluxing agents in order to produce coarse kieselguhr suitable for beer filtration with lowered content of crystalline material.

This study (Project No. 15011BG) was supported by the German Federal Ministry of Economics and Technology (BMWi) via the German Federation of Industrial Research Associations "Otto von Guericke" (AiF) and the Association for the Promotion of Science of the German Brewing Industry

(Wifoe).

P085

Technological and economical reconsideration of depth filtration - Becopad

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Description of topic: 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 the 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. 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 to prove that by re-designing depth filtration the brewers can take advantage of ecological and economical savings.

Materials and methods for data collection:

Results: First results show, that due to several savings (water, energy, labour), filtration cost come down by at least 30%, compared to conventional depth filter sheets.

Discussion: BECOPAD philosophy was part of the oral presentation at WBC in Hawaii, 2008.

What is new - World's first mineral free depth filter medium on cellulose basis. Sustainable, high pure celluloses guarantee purity and hygiene at highest level. New "bepure" technology offers absolute consistent production.

P086

Simultaneous filtration and stabilisation of the beer: Potentiality of Oxidised High Density Polyethylene (PEox) as filter aid

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Description of topic: Nowadays, beer filtration should be performed regarding to economics and be friendly towards environment. The objective of this study was to evaluate the potential of PEox particles as a filter aid media coupled to PVPP and Brewtan® for a simultaneous filtration and stabilisation of the beer.

Materials and methods for data collection: Filter aids were tested on a pilot scale candle filter made of a single candle with a filtration surface equal to 380 cm. Haze sensitive (HA) proteins and polyphenols, as well as chill haze for stability assessment were measured using a Tannometer (Pfeuffer). Regeneration was performed within the mixing tank from the filter using an oxidative detergent and a solution of enzymes.

Results: The filter aid PEox has an average particles size distribution of 37 µm and a shape like "pop corn". The porosity of the media made of PEox is 0.6 compared to 0.85 for kieselguhr. This still allows the material to retain a good amount of haze and yeast cells without clogging, but the expected haze was reached only when using Brewtan®. The use of PVPP mixed to the PEox filter aid (25/75 %) showed best results for filtration due to the shape of PVPP particles and the slight compressibility of the material itself, but its use still could not allow us to reach the expected haze unless Brewtan® was used also during the filtration trials.

Regeneration with oxidative detergent was tested within the tank used for pre-coating followed by separation on the candle. Although the filter aid mixture wasn't as clean as expected, filtration trials carried out after such an operation showed expected results. Enzymatic treatment was also investigated and is under optimisation.

Discussion: There is a real potential for simultaneous beer filtration and stabilisation using a regenerable filter aid such as PEox. Optimisation at industrial scale has still to be done with special regards to regeneration costs and engineering.

What is new - Since decades, kieselguhr as filter aid has been the most common method for beer filtration. However, costs for disposal are increasing and brewers are concerned about replacing kieselguhr filtration by a greener technology. Regenerable filter aid would allow breweries to use the already existing filtration equipment. Filtration trials carried out with PEox in combination with PVPP and Brewtan® showed a real potential of this filter aid for simultaneous beer filtration and stabilisation.

P087

Using microalgae for breweries for CO₂ mitigation and biomass production

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Description of topic: Biological carbon mitigation, in particular engineered closed photosynthesis systems, offer advantages as a viable near-to-intermediate term solution for reduced carbon emissions in the industry sector. Photosynthetic (or "natural" sequestration) systems produce usable byproducts (biomass, biofuels).

Materials and methods for data collection: The advantage of marine microalgae systems for wastewater/flue gas treatment at breweries lies in the potential for high productivity, giving them a small footprint compared to other biological systems, the ability to use otherwise unsuitable water and land resources, the integration with waste treatment and the enhanced production per area, not readily obtained from other biomass sources. Many algal species can grow in brackish and saltwater. This means that the algae technology will not put additional demand on freshwater supplies needed for domestic, industrial and agricultural use.

Results: The objective of microalgae biofixation of CO₂ from breweries is to operate large-scale systems that are able to convert a significant fraction of the CO₂ outputs (flue gas and/or CO₂ from the fermentation process) into biofuels, feed, (bio)chemicals and building materials. For the production of energy, algae can be mixed with brewery sludge and spent grains and converted.

Discussion: Examples are given for low-cost production systems and new developments on biomass conversion which result in higher conversion efficiencies. Phytolutions, Jacobs University and other partners run a project at a German coal power plant from RWE using an 80 000 l reactor to mitigate CO₂ and subsequently convert the biomass into fuels and chemicals. The idea is that only little modifications via R&D are needed to create financially feasible CO₂ mitigation systems for breweries.

What is new - Microalgae offer opportunities for CO₂ mitigation and wastewater/sludge treatment for breweries.

P088

Beer stabilisation by polyamide sorbents

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Description of topic: Insoluble polyamides were first used by Harriss and Ricketts in the late 1950s for beer stabilisation. These investigators showed that Nylon 66 effectively removes anthocyanidins from beer. However, practical use of nylons as a colloidal stability treatment agent has essentially been eliminated, because they cost too much. Therefore in 1960s nylon was replaced by cheaper PVPP. PVPP, which is an insoluble crosslinked form of PVP, represents the most frequently used stabiliser for removal of polyphenols nowadays. In 1980s the research of polyamides for brewing application was done at the Institute of Chemical Technology Prague, where several polyamide sorbents with excellent sorption activity were developed. Nowadays a new polyamide sorbent was developed. This sorbent is kieselguhr covered by a thin layer of poly-6-caprolactame. The sorbent is prepared by in solution polymerisation in the presence of kieselguhr.

Materials and methods for data collection: Sorption characteristics for polyphenols were measured under laboratory conditions and were verified in industrial scale. Trials were done either for single use sorbent type or for reusable one.

Results: Characterisation of the product in dependence on ratio PA/kieselguhr: specific surface 2 - 8 m². g⁻¹, mean particle diameter < 120 µm, pore volume 1 - 7 . 10⁻². cm³. g⁻¹. This sorbent has the same sorptive effectiveness against beer haze polyphenols (calculated on the base of polymer amount) as the equal PVPP dosage. Sorbent has also some sorption activity for proteins, an excellent flow characteristic and a low filter resistance. Moreover this sorbent is absolutely insoluble in beer. It represents a new generation of very effective polyamide sorbent for beer stabilization.

Discussion: Polyamides are true polymers which have all advantages of this category in contrast with the group of cross-linked soluble polymers. These cross-linked polymers are always partly soluble in beer and need swelling before application.

What is new - New generation of polyamide sorbents for polyphenols removal from beer was developed. These sorbents based on kieselguhr and polyamide are absolutely insoluble in beer, need not swelling before application, have excellent flow characteristics and a low filter resistance and remove also part of beer proteins. Their sorption characteristics for polyphenols are similar with PVPP.

P089

Reduction of beer value chain's environmental impact with the use of proline specific endo-protease (PSEP) demonstrated by a comparative Life Cycle Analysis (LCA) screening of PSEP in beer production

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Description of topic: Results of new studies have demonstrated that addition of PSEP (commercial name Brewers Clarex™) also enables shorter cold maturation at elevated temperature while ensuring efficient colloidal stability and maintaining beer quality. PSEP enables brewers to significantly lessen their CO2 footprint by reducing energy cost, water consumption and raw materials usage in beer production.

Materials and methods for data collection: In this LCA all emissions released into the environment and resources consumed along the whole life cycle of beer are added up to produce an inventory list of substances. This inventory is translated with the help of an impact assessment methodology into environmental impacts. This study used two methods: Eco-indicator 99 and IPCC 2001 - for calculating all the impacts and performing sensitivity analysis, respectively.

Results: 1/ Environment profile of stabilizers production: the environmental impact for producing PSEP is about 10 times lower compared to current synthetic stabilizers.

2/ Comparison of environmental cost of producing PSEP against the savings in environmental impact due to the change in beer process: the impact of brewer's beer manufacturing stage is reduced by 5-8% when PSEP is used. The use of regenerated PVPP also could mean savings for the environment however the beer losses involved end up ruling out these savings.

3/ Environmental impact of beer value chain: the total environmental impact represented by 1 million hectoliters beer produced is high. The use of PSEP compared to current synthetic stabilizers reduces

by 2% the total environmental impact of beer value chain.

Discussion: The beer value chain from barley to the recycling of beer bottle can be divided into 4 main stages: suppliers, beer manufacturing, distribution and Use/Disposal. The first two stages account for over 65% of total environmental impact. This study clearly demonstrates that the use of PSEP reduces significantly the suppliers and beer manufacturing stages hence the total environmental impact of the beer value chain.

What is new - World first presentation of Life Cycle Analysis (LCA) screening's results of proline specific endo-protease (PSEP) with current synthetic stabilizers in beer production. Demonstration that the addition of PSEP reduces the environmental impact of suppliers and beer manufacturing stages hence also reduces the total environmental impact of the beer value chain.

P090

Change from a pilot plant brewery to a competence center of brewing and environment technology

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Description of topic: The new challenge in the brewing industry is a process technology, which is able to operate with low investment and low life-cycle costs by producing high quality beers. For the supplier this represents a change of the previous standard Research & Development activity.

The previous main research was located in the production plant. The new, but very important field of activity of R&D, is an exact analysis of the necessary energy and raw material consumptions as well as the analysis and implementation of an energy and resource concept for the brewhouse and the complete brewery, which allows to link all consumers in the brewing plant as well as to optimize operational aspects and the corresponding costs.

In view of this situation, the ZIEMANN pilot brewery, built in 2005, has been designed in a completely new way. The area of the pilot plant has been doubled in order to have sufficient space for the equipment and to develop an environmental technology, in addition to the brewhouse technology especially the no waste concept. This presentation shall give an overview of the new design of the plant, strategic adjustments of the new centre of competence and the first scientific results.

Materials and methods for data collection: Process data collected and evaluated with the tools of Braumat PC S7 and the accompanying MES system which is also installed new. The analyses of wort and beer were done according to MEBAK analysis guidelines. The necessary technical data of the energy process were done according to the corresponding international standards.

Results: New process technology and energy saving technology all over the global steps of the complete brewery plant which are coming out of the development activity of the new pilot center

Discussion: Discussion points:

- water saving due to new cleaning and disinfection system
- energy saving due to:
 - a) new process management with new inline analysis systems
 - b) high Performance Ceramic Recirculation System
 - c) optimization in cooling area

What is new - New brewing process and brewing technology environment strategy

P091

Microbial and enzymatic hydrolysis of brewer's spent grains

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Description of topic: Rising energy costs and limited options for waste disposal organic breweries residues offer an interesting way of energy recovery. Due to the inhomogeneous chemical waste composition and the various solid, pasty and liquid compounds the anaerobic digestion represents an advantageous technology and is in case of wastewater treatment already state of the art. Concerning the chemical composition of spent grains there are still problems in hydrolysing the lingo-cellulose fraction. The performance of hydrolytic bacteria without any pre-treatment is not enough to achieve economically advantageous dwell times. Therefore enzymatic pre-treatment was investigated during a research project which was supported by the Association for the Promotion of Science of the German Brewing Industry (wifoe). Another aim of research was to intensify the microbial activity during hydrolysis.

Materials and methods for data collection: 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 fibre of the residual solid fraction were analysed in duplicate. The composition of the produced hydrolysis gas was analysed by gas chromatography and mass spectrometry (GC/MS).

Results: 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 quickened also the microbial hydrolysis of the lingo-cellulose fraction. By the variation of different fermentation parameters it was possible to define optimal temperature values and optimal space loading.

Discussion: The fact shows that the hydrolytic bacteria are allowed to hydrolyze the easy-degradable substances, while the enzyme is introduced to the system to support attacking the hard degradable substances like lignin-cellulose material. The definition of exact fermentation skills allows exact calculations of pilot plants.

What is new - Previous research topics of anaerobic digestion and hydrolysis of brewer's spent grains focused on mechanical and chemical methods of pre-treatment. A well directed addition of substrate specific enzymes is a new topic in anaerobic digestion of brewer's spent grains. The quantification of hydrolysis gas and its composition was analysed for the first time. Another approach was the definition of optimal temperatures and space loading during the digestion of spent grains.

Abstract - EBC 2009

Category: 10 - Environmental: Utilities and co-products

Keywords: Agriculture;Brewers' yeast;Marketing;Organisational structure;Product development

P092

Marketing co-products to the feed industry

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Description of topic: The management of waste and co-products emissions is moving into the spotlight. Energy efficiency, protection of resources and consumer perception determine the overall strategy of beverage production. To many brewers the incapability of marketing co-products correctly has lead to failure of the financial planning. Others put more efforts into this side-business and became successful players in the market.

Materials and methods for data collection: Observations in the industry

Results: Just as for branding of beers the marketing activities for co-products must follow the same pattern: 1) understanding customers needs, 2) consistency and, 3) differentiation.

The feed industry has faced some massive changes within the last decade. Feed is now regarded as a part of the food chain. Quality and safety standards do not differ between feed and food today. In consequence all suppliers to the feed industry must understand themselves as feed producers.

Animal feeds are designed and calculated based on the specific requirements of livestock and poultry species in each age and stage of production. Variation causes losses in animal performance and is an unacceptable weakness in the highly competitive business environment of feed production. Feed manufacturers tend to limit the use of variable components in animal feed formulation in order to comply with the guaranteed nutrient composition of the finished feeds.

The variations in quality are significantly influenced by (1) the enzymatic degradation during mash preparation, (2) fermentation management and (3) phenomena such as Maillard reactions in the drying process.

Discussion: Finally successful marketing of co-products requires a dedicated sales strategy which is built by skilled personnel, market research and dedicated customer service.

What is new - The focus on by-product quality. Moving away from "waste" towards "a product".

P093

Protect the environment, safe energy and work towards a sustainable future!

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Description of topic: With increased environmental awareness and desire to further reduce costs breweries are focussing on compact possibilities to protect the environment & reduce energy consumption.

Protect the environment: CO₂ as refrigerant

To liquefy fermentation CO₂ gas, a CO₂ recovery plant requires a cooling unit. These units nowadays use typical NH₃ or Freon as coolant. Due to the environmental disadvantages of these cooling agents (NH₃ is toxic and Freon is a moderate greenhouse gas), an alternative, CO₂, is available. CO₂ itself is a refrigerant that has the advantage of being non-corrosive, non-toxic, non-flammable etc., all features which create a safer environment. Compared to NH₃, the great thermodynamic properties of CO₂ enable up to 30% CAPEX and OPEX savings. Safe Energy by up to 60% A CO₂ recovery plant consists of several units, of which CO₂ liquefaction and evaporation units account for a large portion of the plant's energy consumption. With a new energy recovery system, the required energy can be reduced by up to 60%. The two "neglected" energy streams: "heat" released when liquefying CO₂ gas and "cold" released when evaporating liquid CO₂ are optimally applied. Effectively, the new system simultaneously facilitates the Liquefaction of incoming CO₂ gas (from the fermenters) and Vaporization of incoming liquid CO₂ (from the storage tanks). Breweries that expanded their CO₂ recovery system experienced a lower initial investment when applying this energy recovering system. It gives them the opportunity to invest in a plant with a compact, energy efficient and cost effective system rather than large, traditional, energy consuming liquefaction and vaporization units.

Materials and methods for data collection:

- Market research
- Feasible study
- Testing / Pilot
- Partner

Results:

- 2 products
- CO₂ as refrigerant
- LiquiVap

Discussion: Protect the environment, safe energy and work towards a sustainable future!

What is new - The ideal refrigerant: R-744 that has good thermodynamic properties, is non-corrosive and is safe to use for personnel and environment.

A Energy Recovery System that optimally applies two "neglected" energy streams: "heat" released when liquefying CO₂ gas and "cold" released when evaporating liquid CO₂.

P094

The cis-resveratrol/cis-piceid ratio is proposed as a new indicator of the hop freshness

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Description of topic: Callemien et al. (1) mentioned the presence of trans-resveratrol, trans-piceid and cis-piceid in hop pellets. Total stilbene concentrations range from 5 to 16 ppm (1-3). Jerkovic and Collin (3) evidenced the huge impact of the harvest year. No stilbenes were detected in supercritical hop extracts (4). The aim of the present work was to assess the impact of conditioning and storage on hop stilbenes concentrations. American varieties were analyzed through storage in 2 differently conditioned forms (cones and derived pellets) all under protected atmosphere. The stability of trans-piceid and its aglycon was monitored for 1 year under six different model conditions.

Materials and methods for data collection: After removing the resins thanks to cleaning with toluene and cyclohexane, stilbenes were extracted by ethanol:water at 60°C. Stilbenes have been quantified by high-performance liquid chromatography coupled with APCI-tandem mass spectrometry (2).

Results: Stilbenes concentrations of 6 leaf hop varieties and derived pellets were monitored over 12 months of storage. Pelletization induced strong stilbene degradation in some cultivars. Yet pellets emerged as more stable than leaf hop during the first four months of storage. The presence of a new peak identified as cis-resveratrol (up to 1.2 mg/kg in trans-resveratrol equivalents) in all chromatograms of >4 months-stored samples indicates a release from cis-piceid (confirmed in model media). cis-Resveratrol revealed very interesting for assessing hop freshness.

Discussion: trans-Resveratrol, cis-piceid and trans-piceid were found in all samples. After 8 months of storage, the overall stilbene content was decreased in the same range whatever the conditioning. No viniferin was detected in aged hop samples. On the other hand, cis-resveratrol revealed to be release from cis-piceid. The cis-resveratrol/cis-piceid ratio can be therefore proposed as a new indicator of hop freshness.

(1) Callemien, JAFC, 2005 (2) Jerkovic, JAFC, 2005 (3) Jerkovic and Collin, 2007, JAFC (4) Jerkovic and Collin, JAFC, 2008

P095

Origin of hops - determination by isotope ratio mass spectrometry (IRMS)

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Description of topic: The isotope ratio mass spectrometry (IRMS) is a suitable method to determine the origin and the authenticity of agricultural products, foods and beverages. Examples for the application of this technique are asparagus, fruit juice, wine and ham. So the question came up if the IRMS method can be used for hops, too.

By means of the IRMS technique the ratios of the stable isotopes are analysed. The growing regions with their climatic distinctions influence the stable isotope ratios of chemical elements in the biomass of plants. The environmental conditions have also an effect as well as the use of agricultural methods (e.g. fertilization). At work with hops the ratios of the following stable isotopes were investigated: hydrogen, carbon, nitrogen and sulfur.

Materials and methods for data collection: On the one hand hops from the main German growing region (Hallertau) were studied to check the differences in the ratios of the stable isotopes in a limited area. Besides the location the influences of variety and crop year were regarded. On the other hand investigations were carried out on hops from different growing regions. The samples came from the other European and from important global growing regions.

Results: Over the ratio of $^{13}\text{C}/^{12}\text{C}$ and $2\text{H}/1\text{H}$ a differentiation in Hallertau hops is doubtful. But when additionally the ratios of $^{15}\text{N}/^{14}\text{N}$ and $^{34}\text{S}/^{32}\text{S}$ are taken into account the samples can be discriminated according the growing region. The influences of variety and crop year are little but nevertheless they must be regarded. The examination of all four stable isotope ratios allows to

discriminate between each individual growing area.

Discussion: It is shown that the IRMS method can be used to differentiate the origin and the authenticity of hops. The bigger the distance between the growing areas the easier is the differentiation of the hops by means of the IRMS method. The use of the IRMS method as a check instrument is imaginable.

What is new - The isotope ratio mass spectrometry (IRMS) is a highly improved method for the determination of origin and authenticity of agricultural products. This technique is applied to hops for the first time and it is shown that it is suitable. The method allows the discrimination between each individual growing area.

P096

A new approach to the production of isomerized hop extracts

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Description of topic: The present paper relates to a new method for the production of iso-alpha-acids by the isomerisation of alpha-acids in the form of a hop extract.

Materials and methods for data collection: Hop extracts (different varieties, crop 2008), magnesium oxide (food grade), mixing and heating facilities, analysis methods: Analytica-EBC 7.9, 7.10, 7.12.

Results: An efficient isomerization of alpha-acids was achieved after mixing pure resin ethanol-extract with magnesium oxide, filling the mixture into a container, closing the container and heating it up gently, e.g. for 5-10 days at 50°C. Under these conditions all other ingredients of the starting material remained unchanged.

Discussion: The isomerization of alpha-acids prior to the brewing process has been practiced for many years, using well-known hop products such as isomerized pellets and isomerized kettle extracts. The commercially applied processes for both types of products are characterized as follows: The production of isomerized hop pellets is a rather simple process but requires large heating chambers. The isomerization of hop extracts is achieved using alkaline conditions in aqueous solutions, resulting in large amounts of waste water. The goal of the present investigations was to carry over the advantages of the simplicity of isomerized pellet production (mixing hop powders with magnesium oxide, packing the mixture in a foil and heating it up at e.g. 50°C for a period allowing for efficient isomerization of the alpha-acids) to extracts and thereby avoid waste water during production. This goal could best be achieved by using pure resin ethanol-extract as the starting material. The development of this process has resulted in a new type of isomerized hop product.

P097

Radical scavenging capacity of hop-derived products in view of health and brewing applications

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Description of topic: Dietary antioxidants are believed to be effective in the prevention of oxidative stress related diseases (eg. cancer and cardiovascular diseases). Polyphenols are widely recognized as potent antioxidants as they can scavenge reactive oxygen species (ROS). The hop plant (*Humulus lupulus* L.), used in a variety of health applications and indispensable as a beer ingredient, is an interesting source of polyphenolic antioxidants including tannins, flavonol glycosides and prenylated flavonoids. In addition, also hop oil and hop acids (including downstream products) have been reported as potent antioxidants.

Materials and methods for data collection: A range of hop products (including different extracts and downstream products) was provided by Hopsteiner. The peroxy radical scavenging capacity was investigated using the ORAC assay (AAPH as free radical generator, fluorescein as probe, Trolox as reference). The hydroxyl radical scavenging capacity was investigated using a modified version of the ORAC assay (using H₂O₂ and CuSO₄ to generate radicals instead of AAPH). Quercetin and a grape extract containing oligomeric proanthocyanidins (OPC) were used for comparison.

Results: Peroxy radical scavenging capacities of prenylated flavonoids were highly analogous to quercetin and OPC equaling 5-10 Trolox equivalents. The hydroxyl radical scavenging capacities of xanthoflav products was highly correlated with the concentration of xanthohumol. The hydroxyl radical scavenging capacity of pure xanthohumol (> 95%) was about 60 Trolox equivalents which is 10-20 times higher than that of quercetin and OPC. Ethanol extract consistently showed a higher radical scavenging activity than CO₂-extract. Tannin extract proved an efficient peroxy radical scavenger.

Discussion: Xanthohumol containing products showed high radical scavenging capacities, which partly may be mediated by metal ion chelating properties. Apart from their possible health benefits, these products might also contribute antioxidant power during the brewing process and during storage.

What is new - Most antioxidant investigations on hop products have used the DPPH-radical assay. Instead of investigating the radical scavenging capacity, this assay rather provides a measure for the redox properties of a compound, as the mechanism is based on electron transfer rather than hydrogen atom transfer. In our work, we have investigated the peroxy and hydroxyl radical scavenging capacities of a large range of hop products. Xanthohumol proved a highly efficient hydroxyl radical scavenger.

P098

Effect of Bordeaux mixture (copper sulfate) on 4-Mercapto-4-methylpentan-2-one content in hop cones

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Description of topic: 4-Mercapto-4-methylpentan-2-one (4MMP), 3-mercaptohexan-1-ol (3MH), and 3-mercaptohexyl acetate (3MHA), which belong to thiols, contribute to blackcurrant/muscat-like aroma of beers using some USA cultivars such as Cascade cultivar. 4MMP has particularly low threshold value and strongly influences beer aroma. In our previous study, 4MMP existed in the hop pellets cultivated in USA, Australian and New Zealand, but did not in Europe. Then we assumed that the decrease of 4MMP concentration in European hops may be caused by Bordeaux mixture (copper sulfate) which is used in only Europe for protection against downy mildew. Therefore, we conducted the field tests for investigating effects of Bordeaux mixture on 4MMP content. In this paper, we describe the results of the field test.

Materials and methods for data collection: We studied the field test for Cascade in Yakima(USA) where copper sulfate is not used regularly. Frequency of spraying copper sulfate to the test blocks in the fields was as same as in Europe (twice a year). The amounts of thiols (4MMP, 3MH, 3MHA) in the cultivated hop cones and beers using these hops, divalent metal ions in hop cones, esters and terpenoids in beers were quantified.

Results: We observed 4MMP content of hop cones and beers treated with Bordeaux mixture decreased by approximate 25% compared to untreated samples. The copper ion content of treated hop cones was only that of 1/3 to 1/5 of general European cultivars, in contrast that of untreated hop cones was below detection limits. The level of other substances in hop cones and beers were hardly different between treated and untreated samples.

Discussion: We showed that the use of Bordeaux mixture reduced the amount of 4MMP in hop cones of USA, however, not to the levels of commercial European cultivars. The effects of spraying Bordeaux mixture in one year may be weaker than expected. Copper ions accumulated in soil for past years are assumed to affect the 4MMP content in hop cones and beers using these hops.

What is new - The use of Bordeaux mixture reduced the amount of 4MMP in hop cones of USA,

however, not to the levels of commercial European cultivars. The effects of spraying Bordeaux mixture in one year on 4MMP content in hops and beers may be weaker than that of absorbing accumulated copper ions in soil for past years.

P099

Scanning electron microscope (SEM) examination of lupulin glands of different hop varieties

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Description of topic: The flavour active components of hops are the hop resins and the essential oils, which are produced in the lupulin glands. The sizes of the glands are suspected to show a relationship with the content resins and oils as well as with the storage stability of alpha acids. The aim of this study was to investigate the lupulin glands of different hop varieties on one hand to show how they differ in appearance and structure and how homogeneous the glands are within one variety. On the other hand if the size and the structure are in any relationship with the valuable components of hops. Therefore 10 different hop varieties from the USA, Great Britain and Germany were investigated with a scanning electron microscope.

Materials and methods for data collection: For each variety several hop cones were used to place a large number of glands (>500) on SEM stubs. The lupulin containing stubs were immediately freeze dried and then stored in a desiccator. A Joel JSM-5510 (5kV, 400 to 500 times magnification) was used to take images of the lupulin glands. From the images the diameters of 50 different glands of every individual variety were measured and the size of the glands was estimated. Resin content was determined using HPLC methods.

Results: In general the lupulin glands vary extensively in size and structure within one variety. The diameter of the glands varies between 50 µm up to 400µm. And the average diameter of the individual varieties differs extensively as well. A direct correlation between the average size and the alpha acids was not found. For most varieties the glands were found to be completely filled some varieties showed a large percentage of flat and almost empty glands.

Discussion: The structure of lupulin glands even in a single variety differs extensively in their structure and size. This could be a hint that there are different types of glands and it is possible that some containing more resins some more oils. Further research in this field is necessary to confirm this.

What is new -

Abstract - EBC 2009

Category: 12 - Malting: Malt production & quality

Keywords: Antioxidant;Barley;Malt steeping;Polyphenol

P100

Contribution of free phenolic acids and flavanols to antioxidant activity in malting process of barley

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Description of topic: Polyphenols (PF) influence beer quality and sensory stability being involved in

beer hazing. The aim of this study is to evaluate the antioxidant activity (AA) of some PF during malting process of three varieties of barley and, in particular, the effect of different steeping conditions on PF extraction. Moreover the correlation between PF and antioxidant activity during each step of malting has been evaluated.

Materials and methods for data collection: Three different varieties of barley were malted according to standard procedures to obtain a pils malt. Three different pHs for the first steeping were used: 5, 7 and 8. PF were evaluated after silanization by GC-MS analyses. The major peaks identified are *o*-hydroxycinnamic, vanillic, trans-ferulic, *p*-cumaric, caffeic, cis-ferulic acids and the flavanols (-)-epicatechin and (+)-catechin. The concentration of each compound was monitored during malting steps. The AA was measured using the crocin kinetic competition test on Std solutions.

Results: After the first steeping, PF content decreases more at pHs 7 and 8 (22-51%) compared to pH 5 (3-13%): *o*-hydroxycinnamic acid and (+)-catechin are the main PF involved. From steeping to germination the PF content increases for all conditions while, during kilning, the levels of PF decreases. The PF content in malt is influenced by variety and pH used during steeping. In addition, testing the capacity to quench free radicals as an index of AA, PF show a low activity.

Discussion: The solubilisation and the stability of PF depend on the pH and on the chemical structure of PF involved. Highest pH values reduce PF content up to 51%. Moreover some enzymes can be activated at different pH values and this can reflect the highest extractability from kernel structures after germination. The decrease of PF during kilning is due to their degradation or other reactions (e.g. Maillard Reaction). Considering the concentrations, phenolic acids give a low contribute to AA and this underlines the role of other compounds e.g. vitamins, carotenoids, melanoidins.

What is new - The influence of polyphenols (PF) on the antioxidant activity (AA) and their solubilisation in different steeping malting conditions are still not clearly explained. The effect of phenolic acids is too low to justify the high total AA actually due to other compounds. Barley varieties have PF with different chemical structures which are more or less sensitive to pH variations. Therefore barley variety and steeping pH conditions should be evaluated in order to reduce the haze formation in beer.

P101

T2 and HT2 toxins elimination by GRAS micro-organisms in steeping waters

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Description of topic: T2 and HT2 toxins increasingly recovered in terms of frequency and concentration on barley in the fields. Even if these toxic molecules are washed out from barley during the steeping phase, worrying amounts are susceptible to be released under the form of steeping waters, raising environmental and safety concerns. In this context, this work presents an efficient, cheap and robust toxin decontamination approach based on the use of the common enzymatic potential of GRAS microorganisms such as yeasts and lactic acid bacteria.

Materials and methods for data collection: The T2 and HT2 toxins resistance thresholds and potentials for microbial elimination have been determined via solid and liquid microbial cultures with given initial amounts of mycotoxins. Growth, substrates and mycotoxin concentrations were respectively determined by OD measurement, HPLC, and LC-MS-MS.

Results: This work has first allowed the selection of highly T2 and HT2 resistant GRAS strains with toxicity thresholds of up to several thousands ppb of T2 for *Geotrichum candidum* for example. These resistant strains have then been evaluated for their ability to eliminate T2 and HT2. These toxins were effectively eliminated at rates up to 15 ppb/hr or 10 ppb/g of biomass/hr in laboratory conditions. What is more, mycotoxin balances in the broth and at the cells level showed that the main elimination mechanism is biodegradation, cells adsorption or absorption representing only 5 % of the global elimination.

Discussion: This work has demonstrated the feasibility of T2 and HT2 elimination in steeping waters by using very common GRAS micro-organisms. High elimination rates compatible with industrial elimination needs have been observed. However, as the main elimination mechanism is biodegradation and as it can be only partial, the residual toxicity of treated T2 and HT2 solutions

remains to be determined before to go further in the development and optimization of a global microbial elimination process.

What is new - Use of GRAS micro-organisms in aerobic conditions for toxins elimination.
Characterization of the main elimination phenomenon : biodegradation vs adsorption and absorption

P102

Effect of interactions existing between barley dormancy and exogenous conditions of germination on some selected parameters of malt quality

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Description of topic: The aim of this study was to analyse physiological condition of kernels of two barley varieties in the course of dormancy. Analyses were performed on three sampling dates in an interaction with the exogenous modification of intergrain gas produced in the course of malting.

Materials and methods for data collection: Experiments were performed with varieties Jersey (USJ 6.3 p.) and Tolar (USJ 2.1 p.). Standard malting were continuing without a modification of intergrain gas and carbon dioxide was sorpted and ethylene was added in the tested variant. Experiments were performed immediately after the harvest and thereafter at intervals of three and six weeks after the harvest.

Results: Experimental results corroborated physiological differences existing between tested barley varieties. During germination, Jersey variety produced more CO₂ and less ethylene than Tolar (which showed a deeper dormancy). In the course of dormancy, overall CO₂ production increased in both varieties; however, that of ethylene showed a reversed trend. In the course of dormancy, parameters selected for the evaluation of quality of malts produced within three different time intervals improved: activity of α -amylase increased, content of β -glucans decreased and malt modification and homogeneity improved. After a change in composition of the gas environment, the yield of malting insignificantly decreased, while activity of α -amylase significantly increased; the content of β -glucans in malt decreased, malt modification increased and malt homogeneity slightly (insignificantly) decreased.

Discussion: Ethylene and carbon dioxide have influence on the germinating of the grains.

What is new - It was demonstrated that the effect of exogenous conditions of germination (i.e. of increased level of ethylene without CO₂) on yield of malting, alfa-amylase activity, content of beta-glucans in malt and malt homogeneity and modification was significant.

P103

Behaviour of organic radicals in different malt types during the malting and mashing process

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Description of topic: The ESR spectroscopy is a rapid method for detecting ions with unpaired electrons and organic radicals. Besides the liquid measurements the ESR-spectroscopy has been used in the past to control the concentration of organic radicals in malt or green malt, by using Mn²⁺ as an internal standard. A novel method for solids measurement using a new reference signal was applied. Based on this, it was possible to analyse the radical concentration in barley, malt and spent grain quantitatively. It has been additionally used for the investigation of radical concentration in different fractions of different types of malt samples as well as their development during the mashing

and malting process under different technological conditions.

Materials and methods for data collection:

- EPR X-Band Spectrometer,
- pilot malting plant,
- different pilsner, coloured and roasted malt.

Results: The results show that withering and kilning have a major influence on the radical generation in malt. A strong increase during these processes shows high stress conditions and intensive oxidation reactions. Also different radical concentrations located on certain malt fractions in different malt types could be detected. In pilsner malt the highest concentrations were located in the husks fraction and the lowest were found in the endosperm. A correlation between extract yield and organic radical concentration in spent grain with respect to mass was achieved. Coloured and roasted malt contain a higher organic radical concentration and also a different distribution of these radicals in the different fractions. This leads to a higher probability that organic radicals participate in the oxidation reactions during mashing.

Discussion: The correlation between extract yield and radical concentration in spent grain allows to use the new method to investigate the influences of mashing and lautering. When colour or roasted malt is used, more radicals participate in the oxidation reaction during mashing. This is one cause for a lower endogenous antioxidative potential in beer.

What is new - A novel method for solids measurement by using ESR Spectroscopy and a new reference signal was applied. This signal can be detected on the ESR spectrum besides the organic radical signal which allows a better quantitative detection of the organic radical concentration. Based on an established correlation between extract yield and radical concentration in spent grain it is possible to use this method to investigate the influences of the mashing (temperature, time, rests) and lautering processes.

P104

Impact of the steeping process on the modifications of lipid transfer protein (LTP) from malt

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Description of topic: The aim of this work is to assess the impact of the steeping process on LTP modification during malting.

Materials and methods for data collection: In this regard lipid transfer protein (LTP 1) modification was monitored in malt produced through four steeping diagrams leading different moistures.

Results: LTP1, is a major beer protein (50% of beer protein) and contributes to the foaming properties of beer. While the barley protein is unable to form foams the corresponding beer LTP1 display good foaming properties. This is due to both glycosylation and acylation during the malting process and denaturation on boiling during brewing. The modifications of LTP are followed by quantification of acylated LTP and comparison of LTP glycosylation content in malts obtained at different hydration diagrams.

Discussion: Significant differences were observed on the structure and chemical modifications of LTP isolated from malt produced at different steeping diagrams.

What is new -

Abstract - EBC 2009

Category: 12 - Malting: Malt production & quality

Keywords: Barley;Malt quality;Nitrogen;Protein

P105

Protein fractions in two-row barleys as malt quality index

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Description of topic: The composition of the protein fractions is affected by genotype and environmental conditions. The focus is on understanding how albumin, globulin, hordein and glutelin could be an index of malt quality (more than the total protein alone). The effect of different N fertilization levels in two years on protein fractions and the relationships between malt quality parameters and these fractions have been investigated on autumn barley varieties.

Materials and methods for data collection: A field experiment compared three two-row malting varieties, fertilised with two N levels (0 and 150 kg/ha). Protein fractions were sequentially extracted from barley. Barleys were micro malted and malt quality parameters were determined. ANOVA was applied on technological and agronomical factors and simple correlations for protein fractions and malt quality were considered.

Results: There was a significant difference in protein fraction composition. Varieties influence significantly albumin-globulin and hordein level; hordein and glutelin are influenced by N fertilization level and year influences glutelin level. Hordein was negatively correlated with HWE ($r=-.64^{***}$), index refractometric ($r=-.67^{***}$), friability ($r=-.71^{***}$) and positively correlated with viscosity ($r=.48^{**}$), β -glucan ($r=.64^{***}$) and N level in barley ($r=.68^{***}$). Glutelin was negatively correlated with friability ($r=-.44^{***}$) and positively correlated with FAN ($r=.50^{***}$) and N level in barley ($r=.56^{***}$). Albumin and globulin were not correlated with the considered factors.

Discussion: Hordein can explain better than total N in barley, malt quality. High hordein contents are present in hard endosperms causing a not uniform milling with low friability and HWE. In addition, in steeping malting process, the hordein in the sub aleurone region could slow down the distribution of water into the endosperm and low levels of β -glucan solubilise are activated with slow β -glucan breakdown. The glutelin is the main protein fraction involved in FAN production.

What is new - There is a scant information on the role of protein fractions on malt parameters. This work investigates different environmental and agronomical conditions and the responses of protein fractions on malt quality. N fertilisation levels and varieties influence protein fractions. High hordein content is correlated with a low HWE and low β -glucan degradation during malting, with a final negative effect on malt quality and in brewing process; glutelin promotes FAN and can improve yeast performance.

P106

Chasing the premature yeast flocculation factor from barley to beer

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Description of topic: Some of the most difficult and frustrating problems to solve are those that happen only occasionally and have no obvious pattern or cause. One such problem in brewing is when the yeast begin to flocculate before fermentation is finished. Over the years, brewers have asked what causes this premature yeast flocculation (PYF)? We are still waiting for the answer. Today, the most recent research suggests that PYF is connected to malt quality and might possibly be triggered by conditions in the field before the barley is harvested. In this paper we will discuss our work tracking this PYF factor during the malting process. We approached this problem by investigating the link between the malting process and PYF and asking whether malting conditions can be adapted such that a 'PYF barley' can be prevented from becoming a 'PYF malt'

Materials and methods for data collection: Barley samples were sourced from several different areas of UK. The PYF characteristics of these barleys were measured before, during and after malting using our in house PYF testing procedure.

Results: Small scale malting trials confirmed that PYF factor can be removed from barley during

steeping, however the malt can become PYF during germination whether or not it started as PYF barley. Air recirculation during germination decreases PYF. In commercial maltings a PYF barley can result in PYF positive or negative PYF malt according to whether the process conditions were sufficiently aerobic.

Discussion: Despite the large amount of research on this subject, it is still a mystery as to what factors are involved in PYF, and how to find a solution to this problem. Our data suggest that malting conditions could be used to mask the effects of a PYF factor in the malt, with this knowledge can we make PYF a thing of the past? Does the condition during malting exert the same physiological stress as microbial growth? Could the microbiological conditions in the grain be critical in the development of the PYF factor? If so, is it a step that could be controlled throughout malting?

What is new - This study includes novel data from samples taken during the malting process and increases our understanding of the PYF factor in malt. It assists in optimizing malting procedures for grain which may be PYF positive to make malt acceptable for brewing.

P107

Studies of flavour development during malt roasting processes

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Description of topic: Speciality malts can be used to add colour and flavour in the brewing process. Published literature mainly describes the analysis of flavour compounds in roasted malt products, rather than examining how process conditions may be manipulated to influence the volatile profile. Our group has established a rapid on-line APCI-MS technique, capable of monitoring thermal volatile generation in malts in real-time, so that comprehensive studies of the impact of process conditions (time, temperature, humidity) on the malt volatile profile may be undertaken. Here we report results obtained using the on-line technique and compare these with flavour generation profiles obtained from time-course studies during commercial malt roasting operations.

Materials and methods for data collection: The volatile APCI-ions monitored during on-line thermal treatment of barley malt (var. Cocktail) have been assigned to compounds using SPME sampling followed by GC with synchronous dual MS detection (EI/APCI). To check the validity of on-line data, we have conducted time-point sampling during both pilot scale (2 kg) and commercial roasting (2 tonne) operations. Malt samples (12 per run) were snap frozen in liquid nitrogen and subsequently analysed in triplicate for colour, moisture and flavour volatiles by solvent extraction and GC-MS. Duplicate production runs were monitored for i) crystal malt; ii) black malt and iii) roasted barley manufacture.

Results: 13 significant malt volatiles were identified (on the basis of EI mass spectrum and Kovat's Retention Index) which gave 'unique' APCI-MS ions during on-line analysis. Quantitative time-course data were obtained for 15 significant volatiles generated during the manufacture of roasted barley, crystal and black malts on both a pilot and industrial scale.

Discussion: Commercial flavour data will be compared with results from on-line MS experiments conducted over much shorter timescales. Emphasis will be placed on how the volatile profile changes with time and also on the impact of factors such as 'quenching'.

What is new - This work offers insights into the mechanisms of flavour generation in roasted malt products and is unique in comparing volatile generation profiles for three different scales of operation. The on-line APCI-MS technique is novel and is being used to explore the relative significance of different reaction pathways to flavour formation under differing reaction conditions. The ultimate application might be the creation of novel products or optimisation of current practice (energy efficiency).

P108

Effect of water content of barley grains during malting on germination and malt quality

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Description of topic: Barley grains are mostly used for malting, an industrial process which consists in 3 main steps : steeping, germination and kilning. During steeping water content of the grains is increased to 42-45% fresh weight (FW) basis to allow germination. In order to save energy during kilning, it would be important to achieve germination and obtain a good malt quality at lower moisture contents. The aims of the present work were then to determine the lower water content required to allow germination and to investigate the effect of seed water content during the germination phase on malt quality evaluated by the activities of hydrolysis enzymes (alpha- and beta-amylases, beta-glucanases), soluble sugars (maltose, sucrose and glucose) and one of the main component of barley endosperm cell wall (beta-glucans).

Materials and methods for data collection: Experiments were performed with 2 varieties (Azurel and Sebastian). The critical water content (WC) for germination, enzyme synthesis and reserve mobilization was determined by incubating the grains at various WC ranging from 32 to 44% FW at 15°C for 5 and 7 days and at 25°C for 3 days. All the biochemical measurements were carried out using Megazyme kits.

Results: Germination of 90-95% of the seed population occurred at 15°C within 7 days or at 25°C within 3 days when the seed water content was higher than 34-35% FW. In vitro alpha- and beta-amylases activities increased with increasing water content. In contrast, after 7 days at 15°C, beta-glucanase activity reached a plateau already when water content was 36% FW, but its in vivo activity, evaluated by beta-glucan content, required higher water content. For the same temperature sum applied during malting, malt quality was lower at 25°C than at 15°C.

Discussion: Differential sensitivity to water content of the germination process, enzyme synthesis and reserve mobilization was discussed. However, results obtained allow us to suggest a critical water content of 38-39% FM to be able to produce a malt of sufficient quality.

P109

Demand response and dispatchable industrial loads for the use in virtual power plants

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Description of topic: Within the project "Virtual Power Plant" (VPP) RWE Energy AG evaluates the options to bundle and merchandise small power and energy consuming units in the energy market. In a sub-project IER analyses the availability and useability of dispatchable loads in a VPP at the Bitburger brewing plant. Dispatchable loads and storage facilities are checked towards options for their technical and economical applicability in a supra-regional market model of a VPP.

Materials and methods for data collection: The production processes and generation units at the Bitburger brewing plant are analysed with a standardised approach to identify dispatchable loads. The technical, economical and organisational requirements to implement loads in a VPP are analysed. The profitable use of the identified loads is evaluated by computing scenarios with varying energy price levels. With a simulation tool the load shifting options are calculated and synthesised load profiles are created. The total potential of dispatchable loads and storage facilities at Bitburger is estimated. Furthermore different strategies for the use of these loads in a market model of a VPP or in the demand response strategy of the brewing plant are simulated.

Results: For Bitburger brewing plant dispatchable loads of more than 1.1 MW are identified and can be technically and economically used in a market model of a VPP. The results at Bitburger brewing plant can be transferred to other sites in beverage and food industries as the applied processes are similar. In addition the load profile of Bitburger is leveled and the maximum load demand can be reduced. Loads starting from 100 kW have the potential to be utilised within a VPP.

Discussion: The operational requirements of a VPP have to be harmonised with the load demand of

the company in order to ensure the production. The integration of the dispatchable loads and storage facilities in a VPP must be checked under consideration of existing load management systems and possible restrictions from the grid.

What is new - By participating in a VPP a brewing plant can shift loads for several hours. VPP can merchandise these loads at energy markets. Dispatchable loads and storage units open the potential for production sites to play a role in a VPP and to benefit economically. In future the application of dispatchable loads in a VPP can offer new options in energy supply contracts for production sites.

P110

Development of a demonstrator application for the model based weak point analysis of bottling plants

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Description of topic: The efficiency of today's bottling plants is not often higher than 40 to 70 percent. In order to optimize the plants downtime originating components should be identified automatically. For this reason a model based diagnosis algorithm has been developed in an interdisciplinary research project that will be presented at EBC 2009 in the accepted lecture of Dr. Tobias Voigt. For the practical benefit of breweries, the diagnosis results need to be analyzed and visualized in an operator comprehensible way.

This contribution will present a demonstrator application for the automatic weak point analysis of bottling plants. It was developed to show the practical opportunities of this new approach.

Materials and methods for data collection: The model based diagnosis algorithm was implemented into a demo application, to automatically analyze the production data of the bottling plant components. The diagnosis results are stored into a standardized data structure that could be used from several programs for visualization. The presented realization is based on MS Excel. This allows showing diagrams and calculating performance indicators.

Results: The first version of the demonstrator application will be implemented in industrial scale bottling plants in February 2009. A validation through simulation and real data from proved evidence between the automatically diagnosis results and the manually monitored behavior of the plants. Technical downtime reasons can be identified with an accuracy up to 95 percent. With this information a plant operator can quickly take actions to optimize critical weak points.

Discussion: The demonstrator application is a new tool to automatically detect critical technical weak points of a bottling plant. As it is based on a component oriented model based algorithm, it can be adapted to different plant constellations easily. As first planned pilot projects by industrial research partners show, the results will enable affordable diagnosis tools for brewers to raise their bottling efficiencies.

What is new - Current solutions for the data analysis of bottling plants work with statistical evaluation unaccounted for the effect of the efficiency of the whole plant. The model based solution allows a directly connection between a downtime of the plants central aggregate and the originating component. Now it's possible to generate e.g. pareto charts to visualize weak points of the bottling plant automatically.

P111

Quorum sensing of brewery biofilm microbes

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Description of topic: Bacteria are no longer regarded as undifferentiated cells focused on multiplication. Instead it has been shown that cell-to-cell signalling both within and between bacterial species is a widely spread phenomenon, and there is evidence that quorum sensing participates also in biofilm formation. Compounds inhibiting either the synthesis or use of signalling molecules have been detected, and in the future there might be a possibility to interfere harmful biofilm formation of bacteria in breweries by controlling their signalling.

Materials and methods for data collection: Samples were taken from brewery filling machine surfaces and cultivated on Tryptic Soy Agar for total bacterial counts. Colonies from these samples were screened for production of signalling molecules by replica plating using *Chromobacterium violaceum* CV026 and *Agrobacterium tumefaciens* NTL4 bacteria as biosensors. In addition, 27 gram negative bacteria previously isolated from brewery surfaces and identified were screened with the same biosensors.

Results: In over 600 replicated colonies from process samples the most common signalling molecule group were acylated homoserine lactones (AHLs) detected by *A. tumefaciens* NTL4, since 90 colonies produced them. Short chain (C4-C8) AHLs detected by *C. violaceum* CV026 were produced by 26 colonies and long-chain AHLs by 8 colonies. From identified brewery isolates 7 strains produced AHLs detected by *A. tumefaciens* NTL4 and 25 strains produced long chain AHLs, but none of the strains produced short-chain AHLs.

Discussion: Strains producing signalling molecules were detected both from process samples and previously identified brewery isolates, but somewhat surprisingly different groups of compounds were prevalent among the process samples and the isolates. Identification and further studies of positive bacterial strains will give information on their significance and participation in biofilm formation. Ultimately, means for disturbing signalling can be tested.

What is new - Bacteria are capable of signalling to each other for instance in forming biofilms. Disturbance of this signalling may in the future provide novel means to fight against harmful biofilms.

P112

Prediction of flocculation ability of brewing yeast inoculates by flow cytometry, proteome analysis and mRNA profiling

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Description of topic: The ability of brewing yeast to flocculate is an important feature for brewing of qualitatively good beer. Flocculation involves two main cell wall structures, which are the flocculation proteins (flocculins) and mannans, to which these flocculins bind. Unfortunately, in practice the flocculation ability may get lost after several repitches.

Materials and methods for data collection: Flow cytometry was employed to analyze glucose and mannose structures of the cell surface by application of fluorescent lectins. Validation of the expression of the flocculin genes Lg-FLO1, FLO1, FLO5 and FLO9 was carried out using microarray techniques. SDS-PAGE, western blot and ESI-MS/MS analyses served to isolate and determine yeast cell flocculins.

Results: Mannose and glucose labelling with fluorescent lectins allowed differentiating powdery and flocculent yeast cells under laboratory conditions. Using microarray techniques and proteomics, the four flocculation genes Lg-FLO1, FLO1, FLO5, FLO9 and the protein Lg-Flo1p were identified as factors of major importance for flocculation. The expression of the genes was several times higher in flocculent yeast cells than in powdery ones.

Discussion: Flow cytometry is a fast and simple method to quantify the proportions of powdery and flocculent yeast cells in suspensions under defined cultivation conditions. However, differentiation under industrial conditions will require mRNA and protein expression profiling.

What is new - Please note: This paper is accepted for publishing in the journal Cytometry Part A.

P113

Differences in growth behaviour of *Pectinatus frisingensis* isolates in beer

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Description of topic: The growth behaviour in beer of fifty-one *Pectinatus* strains, isolated from eight brewery environments throughout the world or sourced from culture collections, was analysed. The isolates were grouped on the fingerprint types determined in a previous study (by using rep-PCR). The objective of this study was to investigate the relationship between genetic fingerprint types and growth behaviour in beer.

Materials and methods for data collection: All strains were subjected to repetitive-sequence-based (rep) PCR profiling in a previous study. The fifty-one isolates selected to study growth behaviour in beer, represent six out of eleven fingerprint types. OD600 measurements were used to monitor growth in beer.

Results: For isolates from four fingerprint types, different growth curves were observed. The fifth fingerprint type is made up of only one isolate showing a unique growth curve. Within the remaining fingerprint type the five isolates showed similar growth patterns.

Discussion: Isolates from fingerprint types 4a1, 4a2, 4b1 and 4b2, which were genetically indistinguishable according to rep-PCR, showed different growth behaviour in beer. The five isolates that form fingerprint type 4c show the same behaviour in beer; the start time and maximum OD600 were highly similar. Because all isolates used in this study are capable of growing in beer, it seems that besides the starting inoculum other factors are involved. A distinct relationship between genetic fingerprint type and growth behaviour in beer was not shown.

P114

Analysis of metabolic activities of beer-spoilage lactic acid bacteria in chinese beer

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Description of topic: This study shows the first analysis results about typical beer-spoilage lactic acid bacterium and their metabolic activities in final chinese beer, especially the metabolism of sugar and organic acid.

Materials and methods for data collection: Firstly, more than 100 contaminated chinese beer samples were analyzed in the last 12 months; Secondly, the most-appeared contaminated microorganism were identified, which were then inoculated into sterile beer separately. HPLC method is used to analyze the sugar content and the ion chromatographic method was used to analyze organic acids.

Results: *L. brevis*, *L. plantarum*, *L. casei* and *L. acetotolerans* were identified as the most-appeared contaminants. Due to diversification of subtype of *L. brevis*, three *L. brevis* cultures can hydrolyse maltose, which leads to increase of glucose concentrations, whereas other three *L. brevis* cultures metabolize total glucose directly. Maltose and glucose are the major carbon source for *L. plantarum*. It metabolizes also sucrose and fructose. *L. casei* is maltose and sucrose positive. *L. acetotolerans* has never been found in beer before, its metabolic activities of sugar in beer are very low, and besides fructose and glucose it metabolizes only maltose. Almost all lactic acid bacterial fermented citrate.

Discussion: The way of how the beer-spoilage lactic acid bacterial metabolizes rest sugar in final beer depends on the microorganism type. All tested lactic acid bacterial can metabolize the mono carbohydrate. Among organic acids Lactate und Acetate are the major metabolism products. Their

concentrations increase rapidly in contaminated beer. On the opposite, Citrate is metabolized from all analyzed lactic acid bacteria, in some cases, even completely. Typical chromatographs may help to identify beer-spoilage lactic acid bacterial.

P115

Methods for rapid authentication and differentiation of brewing yeast strains

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Description of topic: Yeast quality control is important in the brewing industry. This includes ensuring the right yeast is used in the fermentation, testing to ensure lack of contamination with wild yeasts and checking for any genetic drift in the added yeast during propagation or fermentation that might introduce off flavours or inhibit performance. Restriction Fragment Length Polymorphism (RFLP) or karyotyping is considered as the most effective method to differentiate yeast strains but require considerable knowledge in the molecular biology. Polymerase Chain Reaction (PCR) technology is widely accepted as a diagnostic tool by breweries but has not been applied to brewing strain authenticity testing. So here we present a PCR kit comprising multiple but customised primer sets will provide a rapid differentiation of brewing strains.

Materials and methods for data collection: To test the efficiency of the kit, seven lager strains and six ale strains were incubated for 48 hours, harvested and chromosomal DNA and mitochondrial DNA was extracted separately. DNA extracted from ale and lager strains was used as a template to amplify known sequences using PCR. Resultant sequences were further analysed to confirm the sequence by DNA sequencing. In addition, PCR-RFLP was used to identify sequence differences within lager yeast strains using restriction endonucleases.

Results: Using combined PCR and RFLP we were able to develop specific primers to differentiate ale (*S. cerevisiae*) and lager (*S. pastorianus*). Furthermore, we have also identified primers to differentiate specific lager strains in the University of Nottingham yeast culture collection.

Discussion: PCR technologies have been applied to the detection and identification of non-brewing strain contaminating wild yeast and bacteria but not been used to identify or differentiate brewing yeast strains. This method will enable rapid, accurate, unambiguous and reproducible differentiation of brewing strains and can be easily adapted for use in routine in-house yeast validation.

What is new - Rapid authentication of proprietary brewing strains are very important for a brewer and provide a highly desirable quality assurance tool. Despite this apparent need, there are no proven molecular techniques published to differentiate yeast strains.

Here, we present a molecular tool kit to identify yeast strains with a simple PCR reaction that can be used to validate the right strain within the brewery. The kit will comprise multiple primer sets targeted to specific sequences.

P116

New insights in label removal

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Description of topic: The label come-off time is crucial for the cleaning process in bottle washing machines. If labels come off too late, they will be distributed in the machine and can block caustic and freshwater cones. This will reduce the cleaning efficiency of the bottle washer and will cause an increasing number of rejected bottles, which have to be destroyed or returned to the bottle washing

machine. Either way leads to higher production costs and decreased plant efficiencies. This research examines the come-off time of different paper labels against the influencing factors paper, labeling glue and process parameters.

Materials and methods for data collection: The DIN standard 16524-6 for the label come-off time was modified. Instead of manual gluing on petri dishes, a Langguth E 98/2 labeling machine, in combination with glass cylinders, was used to minimize the personal influence of the tester during sample preparation and to optimize the fluid flow while removing the labels.

Two different kinds of labels, metallized and non-metallized, were used, both with either gravure or offset printing and with optional imprinting and lacquering.

Results: Both printing methods increase the come-off time, but offset printing shows a higher impact. Lacquering labels increases the come-off time only with metallized papers using offset printing. Imprinting papers reduces the come off time with metallized papers only.

With both labels an almost linear effect of the caustic temperature was discovered while different caustic concentration had an effect only on the non-metallized papers using a reduced concentration. However, the major effect on the come-off time of metallized papers was found to be the amount of glue.

Discussion: Metallized papers have a reduced permeability and the glue has to be dissolved from the sides, while non-metallized papers allow the caustic to permeate through the whole surface, which explains the different come-off times of the two paper types investigated. The varieties of the printing methods are caused by the colors being used.

What is new - This research work is the first integral approach to examines the come-off time of paper labels. Unlike DIN standard 16524-6 it also consideres overlapping neck-ring labels with a paper to paper adhesive area.

P117

Practical experiences on the use of UVC-lights as a part of disinfection in filling process

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Description of topic: High hygiene level is a key factor to avoid secondary contaminations in filling process. Disinfection with traditional chemicals requires stoppages in production. Continuous UVC-disinfection can be automated and used during production. UVC has a strong germicidal effect penetrating cell membranes and damaging DNA. Good hygiene practice, cleanability and safety should be considered in design of UVC-lamp installation.

Materials and methods for data collection: UVC-lamps have been installed to filling processes in canning and bottling lines. UVC-disinfection is used for empty cans, bottle crowner and can seaming machines. There are microbiological results from packaged beer and rinsed empty cans. Air rinsing and UVC-disinfection effectiveness has been compared with traditional water rinsing.

Results: Packaged beer results improved with UVC-disinfection in use. Empty can air rinsing and UVC-disinfection is as effective as rinsing with water.

Discussion: A lot of attention is needed to avoid accumulation of micro-organisms and biofilm formation in filling processes. Therefore, filling area design, surface materials, cleaning and disinfection practices are all important factors in overall hygiene level. UVC-disinfection is effective against microbes, it can be used in practical processes and is safe to use, but more experience of material durability is needed.

What is new - Practical application and collected microbiological data of UVC-disinfection in bottling hall.

P118

Filtration and stabilization results from a brewery using a kieselguhr free filter aid

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Description of topic: Kieselguhr filtration is the state-of-the-art filtration technology for beer, with thousands of filter lines in operation globally. Despite discussions about health risk sand disposal costs kieselguhr is the state-of-the-art filter aid. The current consumption of kieselguhr worldwide by the brewing industry is more than 180.000 tons. Disposal costs for the used kieselguhr are an increasing part of total filtration costs, and so brewers are commonly interested since decades in finding a more economical filter aid. Crosspure is a synthetic polymer for filtration and stabilization in one step / one filter. Just like PVPP, Crosspure can be regenerated in a combined regeneration and filtration system with dosing vessel, filter unit and CIP system. For the first time a brewery implemented Crosspure in a industrial scale. Results from the brewery will be presented.

Materials and methods for data collection: Filtration and stabilization results, e.g. pressure drop, haze, shelf life, regeneration process

Results: Replace a kieselguhr filtration in industrial scale by Crosspure, a synthetic polymer for filtration and stabilization in one step / one filter.

Discussion: For the first time there is a technically and commercially attractive alternative to kieselguhr available, which can be used to replace kieselguhr.

What is new - For the first time a brewery implemented Crosspure in a industrial scale. Filtration and stabilization results from the brewery will be presented.

P119

New apparatus and procedure to enhance the utilisation of bitter-acids

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Description of topic: With about 30 % , utilisation of hops in the brewing process is very low. This is due to the fact that most alpha-acids, which cause the bitter taste, are not isomerised and/or extracted out of the added hop products. In addition, alpha-acids can adhere to trub particles and yeasts so that they are separated out of the beer at a clearing process again.

Materials and methods for data collection: Using the knowledge of unit operations in chemical and process engineering, a new approach to enhance the utilisation of bitter-acids was constructed.

Results: With the new invention, alpha-acids are isomerised to an optimum (reachable) level. In the isomerised form, the hop bitter acids show a clearly better solubility in water/wort. In addition, an adhesion is minimized due to the process guidance. The utilisation of hops (bitter acids) is more than doubled while using the invention (>80 %). This is already confirmed in laboratory scale and in a pilot plant.

Discussion: While using the new approach it is possible to clearly enhance the utilisation of hop products. If , for example, the utilisation is doubled, only half of the former used hop products have to be added to wort to reach an equal amount of iso-alpha-acids or bitter units in beer.

What is new - As the invention follows the german purity law, it is now for the first time possible to brew beers with an equal amount of bitter units with a clearly less input of hop products. Thus, enormous savings in raw materials (hops) - and thus in costs - is achieved.

P120

Novel malt-based isotonic beverages

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Description of topic: In recent years a number of novel, innovative beverages have been launched. Due to growing consumer awareness for the negative impact of malnutrition in western countries novel drinks based on natural raw materials have attracted a growing interest. Especially malted grains and natural fruit juices are suitable for the production of such beverages as they are generally considered as positive and healthy food ingredients. A huge variety of malted cereals as barley, the most commonly known, wheat, spelt wheat, rye, oats, triticale and others, e.g. gluten-free pseudocereals may be used.

Materials and methods for data collection: Unhopped wort was produced by using traditional brewing equipment and subsequently fermented by selected strains of lactobacilli (*Lb. amylolyticus*, *paracasei*, and *linderi*). The resulting fermentation products were mixed with fruit juices and natural fruit aroma. After measurement of the resulting osmolality, the beverages were mixed to a osmolality of 290 mosmol/kg. They were isotonic. The osmolality was measured by cryoscopy, the flavor compounds by GC/GC-MS. Tasting was done by a well trained tasting panel.

Results: Traditional tasting schemes for beer or soft-drinks were not suitable for the produced beverages. A new tasting scheme had to be created. The main flavor and aroma attributes of the produced beverages were refreshment, purity of the acidic character, harmony of the used fruits with the lactic acid and stale flavors amongst others. The effect of ageing, pasteurization and flash pasteurization on flavor and flavor stability could be measured with similar lead substances as found in beer like 2-furfural. The produced beverages showed good flavor stability though losses in olfactory attributes occurred during heating and aging.

Discussion: The technology presented in this work offers the possibility to produce novel non-alcoholic beverages with existing brewing equipment. The resulting drinks are rich in natural polyphenols and vitamins and can even be probiotic, if for example *Lb. paracasei* is used.

What is new - No analytical data is available up to now about flavor stability of innovative malt-based beverages. This work provides new knowledge about lead substances for stale flavor in such beverages. Additionally a new tasting scheme considering the special attributes of such beverages is presented.

P121

Industrial results of precoat filtration on a candle filter with regenerable filter aid

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Description of topic: Precoat filtration still is the state of the art filtration technology for beer with thousands of filter lines in operation around the world. Despite all discussion about health risk and disposal problems, D.E. (Diatomaceous Earth) is the state of the art filter aid for precoat filtration. For at least 15 years the experts of the brewing industry have been looking for filter aids, which could replace D.E., so far without much success. Over the last years BASF developed a new, regenerable filter aid called CROSSPURE®, which was thoroughly tested on an industrial scale FILTROX candle filter. The regeneration process is similar to the well established one for regenerable PVPP. Precoat filtration with this new filter aid contributes to the preservation of the environment, because the energy consumption as well as the waste generated is far lower than with any other filtration system.

Materials and methods for data collection: Industrial test in several breweries with a 40 hl/h candle filter, comparison of results with classical D.E. filtration.

Results: Filtration parameters and beer quality are comparable to D.E. filtration, but filtration cost is substantially lower than D.E. filtration and stabilisation.

Discussion: For the first time there is now a technically and commercially attractive alternative to D.E. available, which can be used to replace D.E. Existing filters can be converted from D.E. to Crosspure operation without problems.

What is new - The new SYNOX candle filter was developed for the application of all commercially available filter aids. The new filtration and regeneration process was developed in close cooperation

with BASF.

P122

Kvass - a Russian fermented cereal based beverage

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Description of topic: Kvass or kvas (literally "leaven"; borrowed in the 16th century from Russian квас), sometimes translated into English as bread drink, is a fermented mildly alcoholic beverage made of black or rye bread. It is popular in all ex-Soviet states. Its origins go back 5,000 years to the beginnings of beer production. The alcohol content of Kvass is low (0.05-1.44 %). It is often flavoured with fruits or herbs such as strawberries or mint. This presentation will give an overview on the Kvass technology, which is used in the mentioned countries, combined with different trials for a better understanding of the mostly used raw material rye (*Secale cereale* L.).

Materials and methods for data collection: Brewing has been performed in pilot-scale equipment. Malt and beer were analysed using EBC and MEBAK-methods.

Results: Because there are no typical receipts for Kvass-making all practicable technologies were considered. Nevertheless most of the producers use Kwass-extract, which is made of strongly concentrated Kvass-malt wort. Bread is no longer used for Kwass. The biggest problem of the raw material rye is its high ability for high viscous worts. This can be related to a high content of pentosanes. Further on, oxygen was found with great impact on this mash attribute. Exogenous enzymes solved some problems, but the separation of solid and fluid still depends on the malt and adjunct charge and the lautering system.

Discussion: Kvass is a well known and widespread low alcoholic fermented cereal beverage not only in Eastern Europe. The trials showed that rye, used as adjunct as well as malt, is a raw material with own principles.

What is new - Kvass is a well known beverage but not well presented in the literature. This work gives an overview over the different kvass technologies, their bottle necks and the current evaluated solutions.

P123

Advances in the production of gluten free malt and beer

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Description of topic: The increasing number of people suffering from Coeliac Disease, together with a general growing demand for novel foods and beverages, has given birth to a new market consisting of malts and beers made from gluten free. The technology traditionally used for the production of malt and beer made from barley can not be applied for gluten free cereals. This presentation will give an overview on the changes to the relevant substances taking place during malting and brewing process of gluten free raw material and the difficulties encountered in the process.

Materials and methods for data collection: Malting and brewing have been performed in pilot-scale equipment. Malt and beer was analysed using the EBC-methods. Response surface methodology was used to evaluate the impact of processing parameters on the malt and beer quality. Protein changes were evaluated using the capillary electrophoresis (LAB on the chip), SDS PAGE, 2D gel electrophoresis. Poly phenols, Amino acids and peptides were detected using HPLC. Ultra-structural changes in the grain were visualised using scanning electron microscopy and Laser scanning microscopy.

Results: The areas covered in the presentation are the detailed characterisation of gluten free cereals

and the assessment of these cereals as potential ingredients for gluten free malts and beer. Advanced microscopy has been used to determine the ultra structural changes during the malting of gluten free cereals, where as the proteomic approach was used to explain the protein changes taking place during malting. A detailed nutritional analysis of the malts and beers which includes the impact of processing on the antioxidant capacity, poly phenol content, dietary fibre and mineral content and mineral availability will be shown.

Discussion: This understanding of the different characteristics of the possible raw materials and changes during the malting and the brewing process will allow us to generate concepts to produce gluten free beers with traditional processes and raw-materials.

P124

Development of a hops kettle addition extract to replace aroma hop pellets

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Description of topic: Aroma hops in cone or pellet form are added to impart hop aroma and flavor character to beer. However, this can suffer from low utilization of flavor compounds, high cost, limited availability or variable hop quality. The uncoupling of bitterness and hop aroma and flavor character provides greatest flexibility for the brewer. In this study, we describe our work to develop a non-acidic kettle addition hop extract to replace the hop aroma and flavor character (independent of bitterness) from Saaz hop pellets in a European style lager.

Materials and methods for data collection: Beers made with aroma hop pellets were compared to beers brewed with enhanced non-acidic resin extracts (alpha and beta acids removed) plus independently controlled isomerized alpha acids. Stir bar sorptive extraction gas chromatography-mass spectroscopy (SBSE/GC/MS) was used to identify and quantify key hop components. Hop extracts from generic hops were naturally enhanced with hop-derived oils and standardized to shift the ratios of peaks more towards that of aroma hop pellets. Trained sensory panels were also conducted resulting in descriptive flavor profiles and differences. Analytical and sensory data were correlated using chemometric analysis, specifically principle components analysis (PCA) and partial least squares regression (PLS).

Results: GC/MS concentrations of linalool (10-100 ppb) and humulene oxides (10-50 ppb) were strongly correlated to flavor differences between beer brewed from pellets and different amounts of hop extracts. In a triangle test, no difference was noted ($p>0.05$) between beer brewed with hops pellets and beer replacing a portion of the hops pellets with a kettle addition extract.

Discussion: Hops kettle addition extract is a commercially available, cost effective option for replacing aroma hops.

P125

Ganoderma lucidum - medical mushroom as a raw material for beer with excellent sensorial and pharmacodynamic properties

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Description of topic: Lengthy, Reship, Yogi, "Elixir of life", "Food of Gods", are the synonyms for Ganoderma Lucidum, a woody mushroom highly regarded in Far East folk medicine. It is broadly used in the belief that it boosts up the immune system and treats various human diseases, such as hepatitis, hypercholesterolemia, asthma, allergies and even cancer. The paper gives an overview of use of Ganoderma as a source of bioactive compounds for functional drinks based on beer. It deals

with determinations of main bioactive substances contents, potential therapeutic action and sensory evaluation of the final products obtained by ganoderma and different medical herbs used for sensory adjustment.

Materials and methods for data collection: Dry body of *Ganoderma lucidum* GI-1 was extracted in ethanol, filtered and concentrated in vacuum. Artichoke, linden, ginkgo and green tea tinctures for flavoring were prepared according to Pharmacopoeia. Alcohol extracts in different dosages were added into commercially produced standard lager beer. The final products were sensorial evaluated by a panel of ten tasters. Active ingredients were qualitatively and quantitatively analyzed using LC/MS on an Agilent MSD TOF coupled to an Agilent 1200 series HPLC. Mass spectra were acquired using an Agilent ESI-MSD TOF. Chemical shifts are given on δ scale relative to TMS as internal standard.

Results: Sensory evaluation showed that Ganoderma could give the beer with very good properties. Depending on dosage, combination of bitter constituents derived from hop and ganoderma give specific more or less pleasant bitterness. Products are enriched in bioactive components extracted from ganoderma: triterpenoids, polysaharides and lectins thanks to whom Ganoderma possesses its pharmacodynamic properties.

Discussion: Regarding fullness, taste and flavour new products can be similar to starting beer. Freshness, aroma and even bitterness can be modified with artichoke, linden and ginkgo extracts. Regarding the action of bioactive compounds such beers can be used by quantum satis principle.

What is new - Enriching the commercial standard beer with alcohol extract of *Ganoderma* a pallet of new products can be obtained. With pleasant bitterness, more or less intense, freshness, fullness, taste, flavour and alcohol content these products can satisfy different target groups of consumers, especially those looking for something new and something with proved medical and therapeutic experience in folk medicine.

P126

Critical factors at mashing-in influencing lipid oxidation

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Description of topic: Lipid oxidation takes place during the whole malting process and is known to be relevant for the early appearance of stale and oxidised flavours in pale lager beer. The membrane bound lipoxygenase (LOX), still present in kilned malts, potentially catalyses the formation of trihydroxy fatty acids (thfa) at mashing-in. Considering the relevance of thfa for the final flavour stability of pilsner beers, the objective is to restrict enzymatic lipid oxidation especially at the start of the wort production in order to control the concentration of thfa in the resulting wort.

Materials and methods for data collection: Formation and/or release of thfa has been studied on lab scale by varying mashing-in conditions (temperature, pH). Subsequently 50 kg pilot brews were performed. Thfa concentrations were determined by GC-FID. The potential LOX activity in malt has been measured spectrophotometrically after fine milling, the use of an extractant and linoleic acid as substrate.

Results: On lab scale, mashing-in at 45°C results in a large increase of thfa, higher after fine milling than after coarse milling. At 70°C and pH 5.0, only a small, negligible increase of thfa is observed, independent on fine or coarse milling.

On pilot scale, mashing-in at $\geq 63^\circ\text{C}$ and pH 5.2, only 2-4 mg thfa/L at 12°P are detected in the pitching wort, which is mainly a concentration originating from the malt used. Even using malt with high potential LOX activity, the concentration of thfa in the pitching wort never exceeds 4 mg/L, independent of the milling size. If mashed-in at lower temperature and high pH, concentrations of 8-12 mg thfa/L are detected.

Discussion: Combining higher temperature and lower pH at mashing-in leads to smaller thfa formation, especially due to efficient inhibition of LOX activity. These mashing-in conditions clearly result in pale lager beers with increased flavour quality and stability, as determined by chemical-analytical as well as sensory evaluation.

What is new - Next to LOX and other oxidative enzymes present in the malt, also lipases play a crucial role in the appearance of fatty acid oxidation aldehydes. By choosing correct mashing-in circumstances residual LOX can be effectively inhibited. This is always accompanied by restricted formation of thfa during mashing, indicating the importance of LOX in fatty acid oxidation. Mashing-in under these conditions has no negative impact on saccharification and FAN levels and can improve lauter performance.

P127

Influence of mashing diagramme on arabinoxylan hydrolysis

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Description of topic: Non starchy polysaccharides are the main constituents of endoperm cell walls and aleurone layer. These substances are b-glucans and arabinoxylans. Arabinoxylans that represent 7 to 11% content of the barley grains are not hydrolysed during malting process. These malt substances are known to be implicated in wort viscosity, mash and beer filtration problem. The purpose of this study was to determine the impact of mashing diagramme.

Materials and methods for data collection: Commercial and pilot malts.

Mashing and wort production were carried out on Tepral filtration system

Temperature of proteolytic step: 45, 50 and 55°C

Arabinoxylan was determined by IFBM method

Conventional analysis were carried out according EBC methods

Results: Experiments carried out with malts with different arabinoxylans content clearly showed that the optimum temperature to hydrolyse arabinoxylan is 50°C. Lower and higher temperature led to higher arabinoxylan content in the wort. These results were in connection with the optimum temperature activity of endogenous malt arabinoxylanase. Lower arabinoxylan content in wort came with a lower viscosity and less mash filtration time.

Discussion: Arabinoxylan that is the main polysaccharides in malt and involved in wort viscosity and filtration performance can be well hydrolysed during mashing if the right temperature is applied.

What is new - This work clearly showed that the temperature at the proteolytic step is important if brewer want to hydrolysed malt arabinoxylan without the use of exogenous enzyme.

P128

Impact of cell wall mannoproteins on flocculation of *S. cerevisiae* and *S. pastorianus*

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Description of topic: Process developments require an understanding of yeast responses to changing environments. Whilst it has been previously demonstrated that freshly propagated yeast ferment and flocculate poorly compared to cropped slurries of generation 2 or more the reasons for these differences have not been elucidated. This poster will provide a basis for this frequently observed phenomenon and indicates the means by which flocculation competence may be regulated in full scale fermentations.

Materials and methods for data collection: The coordinated response of brewing yeast to the changing pH, thermal and gaseous environment during brewing fermentation has recently been demonstrated using genome wide expression profiling. The role of CWP, DAN and TIR gene encoded proteins in flocculation has been demonstrated by assessment of *S. cerevisiae* wild type BY4741 and corresponding deletion mutants for Helm's test sedimentation.

Results: We recently established that brewing yeast cell wall composition modifies in response to

changes in fermentation gaseous environmental conditions modifying flocculation performance.

Discussion: This poster has considered the functional role of three gene families which demonstrate modified gene expression during fermentation: the Cell Wall Protein (CWP), Delayed Anaerobic (DAN) and Tip Related (TIR) genes. These data suggest that cell wall mannoprotein expression influences flocculation onset and maintenance.

P129

Zinc supplementation to multi-brew fermentations and zinc toxicity

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Description of topic: This study builds on work presented previously at the World Brewing Congress 2004 on the best way to add zinc to wort. The current study deals with the addition of yeast to multi-brew fermentations and zinc toxicity.

Materials and methods for data collection: Fermentations were performed in 2L temperature controlled tall tubes.

Results: The entire zinc requirement for a multibrew fermentation could be added to the wort which is also pitched with the entire yeast population required for the fermentations. The levels of zinc toxicity were determined.

Discussion: In multibrew fermentations, all the yeast required for the fermentation is added to the first brew. This is based on good microbiological practice and operational expedience. For similar reasons it would make sense to add the entire requirement of Zinc to the wort at the same time as the yeast. The risk in this is zinc toxicity. This was put to test in this study.

What is new - Data on zinc toxicity and fermentation rates under different concentrations and addition regimes of zinc.

P130

Yeast vitality determination based on intracellular NAD(P)H fluorescence measurement

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Description of topic: In this work a cell vitality assessment method based on on-line intracellular fluorescence measurement is presented. The intracellular NAD(P)H fluorescence of a yeast suspension is recorded during transition from aerobic to anaerobic conditions and the output signal is evaluated as a measure of yeast vitality (quality). This method showed good correlation even with low dead cell numbers where the acidification power test failed.

Materials and methods for data collection: Information on yeast vitality was obtained from on-line recorded signal of intracellular NAD(P)H fluorescence during an aerobic-anaerobic (AA) transition in cell suspension. This was ensured by sparging either air or nitrogen into yeast suspension. The yeast vitality was expressed as a relative increase of NAD(P)H fluorescence (rel. FI) between aerobic and anaerobic phase.

Results: The applicability of the intracellular NAD(P)H measurement during AA transition for yeast vitality monitoring was verified during experiments mimicking real storage conditions of pitching yeast in breweries with cells exposed to starvation and gradual loss of viability. During storage the deteriorating yeast viability (3-9 % dead cells) was tracked by rel. FI determination and acidification power (AP) test. The comparison of rel. FI with AP tests came out clearly for the benefit of FI measurement as it is shown by the correlation coefficients. Simultaneously, the time and labour

requirement of rel. FI determination is comparable with the AP tests while both are faster and less labour demanding than cell culture based methods.

Discussion: The quality of brewing yeast is known to influence the fermentation performance and product quality. Although several quantitative methods have been developed to measure cell vitality, none of these has found wide application in industry. In this work, the intracellular NAD(P)H fluorescence was applied for the measurement of microbial activity. The deterioration in yeast viability was accompanied sensitively by decreasing NAD(P)H fluorescence.

What is new - The methods of yeast vitality estimation developed so far have not met the requirements for routine applicability in brewing industry. This work deals with a novel method of yeast vitality determination. The method based on NAD(P)H fluorescence measurement during aerobic-anaerobic transition well reflects the decreasing viability of yeast. From this perspective, the proposed method outperformed both the acidification power test and the culture based specific growth rate determination.

P131

Predicting lager yeast petite formation

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Description of topic: 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 in petite mutations. Eventually this can lead to aberrant fermentation profiles and impaired product quality. Certain strains have an inherent resistance to petite formation. In this paper, the rationale for that resistance is discussed in the context of mtDNA copy number and sequence. This paper will also consider how petites form, when in the process they form and how their formation may be predicted using a rapid test.

Materials and methods for data collection: Using PCR we have developed an assay for the measurement of mtDNA copy number and genome integrity. Using this assay the role of oxidative, ethanol and acetaldehyde damage on the potential of yeast strains to form petites has been assessed. We have applied the assay to cropped yeast from brewery fermentations to elucidate process hotspots for the formation of petites.

Results: The frequency and propensity of yeast to form mitochondrial mutants during serial repitching was determined. Stresses that are encountered by yeast during fermentation and yeast handling have been examined to assess their impact on mtDNA copy number and genome integrity. We have developed a rapid test kit to predict that detects mitochondrial DNA instability before petite formation is evident.

Discussion: Understanding the degradation of mtDNA copy number and integrity during fermentation and yeast handling provides key performance indicators of individual strains. It is proposed that mtDNA copy number is directly linked to the potential of brewing yeast strains to resist key stresses that are increased during high gravity and extensive serial repitching regimes. Furthermore, we propose that selection of brewing strains that are fit for purpose should include an analysis of mtDNA stability.

What is new - Brewing yeast genome stability during fermentation and yeast handling is a key issue for brewers. In this paper the importance of mitochondrial DNA copy number and sequence for the propensity of lager yeast strains to form petites will be presented. This is new data not presented or reported previously. Furthermore armed with this knowledge a novel genome stability test has been developed to permit brewers to assess the potential of strains to form petites. This technique is entirely novel.

Dry yeast - myths and facts

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Description of topic: There are many applications for active dry yeast (ADY) in brewing, depending largely on the scale, type and products of individual breweries. Uses include replacing propagated yeast, seeding propagators, fermenting small batches of beers and bottle conditioning. Despite the range of applications, as well as other characteristics such as long shelf life, until recently multi-national brewers have been reluctant to employ dry yeast. This is often due to the fact that there are many misconstrued beliefs surrounding the use of ADY in brewing. Such ideas include the manner in which ADY should be rehydrated and its use during fermentation. For example, a recently conducted survey indicated that many brewers believe that ADY cannot be serially repitched. Here we attempt to remove some of the myths behind the use of ADY and demonstrate that ADY can be used in a similar fashion to propagated slurries.

Materials and methods for data collection: The fermentation performance of wet and dry yeast over the course of 5 serial repitchings was analyzed using 8.5 liter fermenters. Each fermentation was monitored for sugar utilization and the production of flavor compounds. Yeast cultures were monitored for viability and the accumulation of a range of genomic and petite mutants over time.

Results: The results indicate that a specific rehydration temperature and agitation protocol should be used to ensure fermentation performance. Analysis of chromosomes, inter- σ region variability and Ty element translocations show that ADY cultures are stable during serial repitching. Fermentation data indicated minor differences for the first fermentation using both wet and dry yeast. Subsequent generations produced a consistent product irrespective of the yeast source.

Discussion: ADY is not identical to propagated yeast and should be treated and handled accordingly. However, the data presented here demonstrates that ADY can be used for serial repitching without any detrimental affects to the yeast culture, fermentation performance or final product quality.

What is new - Here we provide novel data regarding the phenotypic and genetic stability of wet and dry brewing yeast cultures during serial repitching. In addition we describe a rehydration regime designed to optimize the viability of yeast and ensure the performance of ADY for the production of beer.