Abstracts from Other Journals

This section contains summaries of recent papers published in a number of other Journals considered of interest to our readers, as well as a selection of patents that have been applied for or recently granted. If you would like to serve as an abstractor for the Journal of the Institute of Brewing, please contact Richard E. Wheeler.

R.E.W.

ABSTRACTORS FOR THIS ISSUE

G. Bathgate, I. Russell, G. Stang, G.G. Stewart and R.E. Wheeler

1) Raw Materials – Barley

Fusarium species synthesize alkaline proteinases in infested barley. A.I. PEKKARINEN, T.H. SARLIN, A.T. LAITILA, A.I. HAIKARA and B.L. JONES (*Journal of Cereal Science,* 2003, 37(3), 349–356).

Barley that is infested with Fusarium head blight (FHB), is not suitable for malting and brewing as it may contain mycotoxins, and also has unacceptable malting quality. Fungal proteinases are often involved in plant–microbe interactions, but very little is known about the enzymes produced by FHB. The authors have previously shown that one plant pathogenic fungus (*Fusarium culmorum*) produces subtilisin- and trypsin-like enzymes when grown on cereal protein.

Field grown barley was infested with FHB as the heads emerged, in an effort to establish whether or not such proteinases were synthesised in vivo. As the grain developed, extracts were prepared and the proteolytic activity was measured. The infested barleys were found to contain both subtilisin- and trypsin-like activities. These extracts strongly reacted with antibodies prepared against the two *Fusarium culmorum* proteinases generated in laboratory cultures, showing the enzymes from the infested barley and from the laboratory cultures to be the same. These enzymes were found to readily degrade barley grain storage proteins (C and D hordeins). The Fusarium proteinases are thought to play an important role in the infestation, but exactly how they function is still not clear.

G.B./R.E.W.

Limit dextrinase in barley cultivars of differing malting quality: activity, inhibitors and limit dextrin profiles. H.A. ROSS, J. SUNGURTAS, L. DUCREUX, J.S. SWANSTON, H.V. DAVIES and G.J. McDOUGALL (*Journal of Cereal Science,* 2003, 38(3), 325–334).

Four barley varieties of different malting quality (Static, Chariot, Optic and Hart) were analysed for Free and Total Limit Dextrin (LD), during malting. Total LD activity levels in Static and Chariot were 4 to 5 times higher than in Optic and Hart 2 days after completion of steeping, with Free LD levels also highest in Static and Chariot (nearly 35% of the total LD level). Free LD rose to less than 15% of Total LD in Optic and Hart. Lower levels of Free LD activity was not however reflected in a higher level of branched dextrins in the malt hot water extracts.

The variation in the proportion of Free LD to Total LD activity between the varieties may be related to the presence of inhibitors of LD. Protein extracts made from the malts were found to inhibit exogenous partially purified LD activity by more than 80%. The varieties Static, Chariot, and Optic lost most of the inhibitory activity after 3 days germination, but Hart retained the inhibitory activity up to 5 days. The availability and effectiveness of LD during mashing is discussed.

G.B./R.E.W.

Evaluation of malting barley quality using exploratory data analysis. II. The use of kernel hardness and image analysis as screening methods. J.P. NIELSEN (*Journal of Cereal Science,* 2003, 38(3), 247–255).

Image analysis and hardness analysis of barley kernels has been used to predict malting quality, using 15 spring and 10 winter barley varieties grown in Denmark. The barleys were micromalted, mashed and analysed for 13 quality parameters using standard EBC methods. A selection of barley samples was analysed using two single kernel instruments:1) the Foss Tecator "GrainCheck" recording the parameters (width, length, volume, area, roundness and total light reflectance), and by 2) the Perten System 4100 (recording single kernel hardness and weight). A total of eight variables from single kernel analyses were used in two different ways; either as means and standard deviations, or as histogram spectra representing 250 kernels from each barley sample.

Using the two methods it was possible to obtain Partial Least Squares Regression (PLSR) models for the structural and physical part of the malting quality complex associated with malt modification. As anticipated, it proved impossible to predict the biochemical parameters associated with nitrogen profiles and enzymatic activity. The best model was achieved for the content of (1→3, 1→4) β D-glucan in the barley.

The hardness of the barley kernel was found to be the most important variable for describing malting performance. The non-destructive image analysis yielded useful morphological data, which was used to improve the calibration models based on hardness alone, and subsequently

revealed some useful malting quality information. The brightness of the kernels was the most important GrainCheck variable, although kernel size and shape were also associated with malting performance.

The utilisation of the single kernel readings (as histogram data), when compared to mean and standard deviation, did not provide any additional information on predicting malting quality parameters.

G.B./R.E.W.

Determination of the molecular weight of barley β-glucan using intrinsic viscosity measurements. Z. BURKUS and F. TEMELLI (*Carbohydrate Polymers,* 2003, 54(1), 51–57).

Determination of polysaccharide molecular weight (MW) usually involves specialized equipment, extensive sample preparation and equipment calibration. Barley β-glucan gum (BBG) was extracted at laboratory (LAB) and pilot plant (PP) scale. Its MW was determined using [η] at infinite dilution. Solutions of β-glucan standards (32–443 K), and PP and LAB gums, were prepared at different concentrations (0.025–0.20% w/w) and viscosity was measured at 25.8 s^{-1}. The intrinsic viscosity was calculated from linear and exponential extrapolation of reduced viscosity. PP and LAB gums had MW of 198 and 598 K, respectively. Determination of MW through [η] measurements is a simple method for characterization of β-glucan.

G.B./R.E.W.

Starch synthesis in the cereal endosperm. M.G. JAMES, K. DENYER and A.M. MYERS (*Current opinion in Plant Biology,* 2003, 6(3), 215–222).

The pathway of starch synthesis in cereal endosperm is unique, and requires enzyme isoforms that are not present in the other cereal tissues, or even in non-cereal plants. Recent research on the functions of individual enzyme isoforms has provided insights into how the linear chains and branch linkages in cereal starch are synthesized and distributed. Genetic analyses have now led to the formulation of models for the roles of de-branching enzymes in cereal starch production. These reveal pleiotropic effects that suggest that certain enzymes may be physically associated.

G.B./R.E.W.

2) Brewing – Brewhouse

Hydroxy fatty acids as indicators for ageing and the influence of oxygen in the brewhouse on the flavour stability of beers. S. WACKERBAUER, S. MEYNA and S. MARRE (*Monatsschrift für Brauwissenschaft,* 2003, 56, No. 9/10, 174–178).

The concentration of oxygenated fatty acids, especially of trihydroxy fatty acids, increases noticeably during the storage of the brewing raw materials barley and malt. Measurement of these lipid oxidation products could therefore contribute to the detection of barley and malt freshness, and could additionally be a helpful tool for an assessment of storage conditions. The influence of oxygen in the brewhouse on lipid oxidation and flavour stability was measured using a number of different pilot plant brews. A noticeable connection to brewhouse conditions was found, but in some cases this effect was overlaid by yeast and fermentation influences.

G.S./R.E.W.

3) Brewing – Fermentation

Measures to improve long term stability of main fermentation with immobilised yeast. K. WACKERBAUER, A. LUDWIG, J. MÖHLE and J. LEGRAND (*Monatsschrift für Brauwissenschaft,* 2003, 56, Nr. 11/12, 210–215).

Although immobilised main fermentation has clear advantages, insufficient long term stability of product quality is the main reason that it is still awaiting its breakthrough. Immobilised main fermentations, on the pilot scale, with varying reactor types, carrier materials and aeration rates were completed, in order to evaluate measures to improve long term fermentation stability. A comprehensive set of data including beer analyses, taste testing and analyses of yeast vitality during fermentation, were collected. It was shown that long term stability is manageable, and a toolset of technological measures is presented to overcome the existing limitations of this technology.

G.S./R.E.W.

4) Beer – Filtration

Importance of deep bed filtration during kieselguhr filtration (Part 2). K. HUSEMANN, F. HEBMÜLLER and M. ESSLINGER (*Monatsschrift für Brauwissenschaft,* 2003, 56, No. 9/10, 152–160).

This second paper relating to the importance of deep bed filtration during kieselguhr filtration deals with the impact of particle size distribution properties of different kieselguhr varieties, on beer particle separation in the filter cake. For these purposes a beer sample with bimodal particle size distribution was filtered using different kieselguhr varieties. The particle size distribution of kieselguhr samples was modified using upcurrent classification and these were subsequently used in the filtration study. The analysis resulted in a high differentiation of particle size distribution of the samples. The poorly filterable beer showed a bimodal particle size distribution with two peaks in the range of 0.8 μm and approx. 6 μm. The readily filterable beer showed a peak in the range of 6 μm (the characteristic particle size of yeast cells). It can be shown that particles of different size can be separated from beer using different mechanisms of kieselguhr filtration: via surface filtration and via deep bed filtration. The particle size distribution, and the surface condition, of the kieselguhr samples impact the separation process. (This study is a continuation of a joint project of the TU Bergakademie Freiberg with the Freiberger Brauhaus AG.)

G.S./R.E.W.

5) Beer – Analysis

The gelation of (1→3)(1→4)-β-D-glucans. C. CLASEN and W.-M. KULICKE (*Monatsschrift für Brauwissenschaft,* 2003 56, No. 9/10, 161–170).

The gelation of (1→3)(1→4)-β-D-glucans in non-filtered beer leads to clogging of the filter media during the beer-brewing process and increased filter media usage, reducing the beer output and increasing production costs. New investigations of the gelation mechanism show different types of gelation. A spontaneous gelation occurs independently of the molar concentration above a critical

glucan concentration (c = 1%) and follows an athermic kinetic with an at least two-dimensional seed growth. The formation of the gel seeds at suitable association points is much faster than the regular gelation. In general, the speed of gelation is controlled by the low molar mass fraction (M_w < 100 000 g/mol) of the glucan. A gel seed formation occurs also below the critical concentration of the spontaneous gelation. However, below the critical concentration of 1% an overall gelation of the solution with the formation of solid gel particles is only possible for high molar mass fractions of the glucan (M_w > 100 000 g/mol) and by an additional increase of the contact rate, for example via shear forces.

An analysis of the oligomeric composition of enzymatic degradation of glucans allowed the formulation of a new gelation mechanism via regular cellotriose units along the glucan backbone in contrast to the widely assumed association via long cellulose-like units.

G.S./R.E.W.

Concentration calculation of beer with linear density formulas. K. TREIBER (*Monatsschrift für Brauwissenschaft,* 2003, 56, No. 9/10, 180–183).

For the determination of original wort gravity within the range of 13 to 20 (w/w) % there is among other things a well-known mathematical linear formula with an accuracy of ±0.03 (w/w) %. This formula can be used for the determination of original wort gravity within a range of 10–21 (w/w) %, but with a reduced accuracy of ±0.1 (w/w) % or by applying a simple correction to the formula of ±0.07 (w/w) %. Through further adjustments of this mathematical formula and via range containment, it is possible to determine the original wort from the density d20/4 over the density dT with an accuracy of less than ±0.01 (w/w) % within the range of 10 to 18 (w/w) %. Remarkably enough this does not involve complicated arithmetic operations, but rather the fact that this correlation can be described with linear functions.

G.S./R.E.W.

6) Beer – Flavour and foam stability

Influence of pre-isomerised hop on taste and foam stability. M. CVENGROSCHOVÁ, G. ŠEPEL'OVÁ, and D. ŠMOGROVICOVÁ (*Monatsschrift für Brauwissenschaft,* 2003, 56, Nr. 11/12, 206–209).

Addition of hop products into a wort boiling process supplies colloidal, biological, organoleptic and foam stability as well as effects on bitterness, aroma, colour and gushing. Nowadays hop pellets, extracts and pre-isomerised hop products are used. Using of pre-isomerized hops makes manipulation easier because of lower dosing, and increases utilisation. The price level of the hops compared with HHT hops is lower by one half on average, and bitterness analytical results are reached on the same level. During measurement of bitter substances by the BU method there were no differences in values, but isohumulone measurement shows that perceived taste bitterness is higher (equivalent to BU × 1.2). There was no influence either on foam stability or on gushing creation. Some negative taste properties where found during organoleptic tests, mainly in beers with higher hop content, with "tart" and "higher hoppy" and "ester" tastes noted as negative taste properties.

G.S./R.E.W.

(R)-Linalool as a key flavour for hop aroma in beer and its behaviour during beer staling. D. KALTNER, M. STEINHAUS, W. MITTER, M. BIENDL, and P. SCHIEBERLE (*Monatsschrift für Brauwissenschaft,* 2003, 56, No. 11/12, 192–196).

It was shown that the chiral distribution of linalool in aroma hops, ranging from raw hops to all conventional hop products, stays almost stable at 94% R-linalool. In beer considerably lower amounts of R-linalool are found. A racemisation during the brewing process is thought to be the cause. The degree of racemisation depends considerably on the type of hopping.

In the course of beer staling racemisation continues with the overall amount of linalool remaining constant. As a result, a flavour loss is detectable by organoleptic testing. A main factor for the transformation from (R)-linalool into (S)-linalool during beer staling may be the pH.

G.S./R.E.W.

7) Microbiology

Combined rapid detection method using micro-sieve filtration and PCR-analysis for direct determination of contaminant micro-organisms in beer. K.-J. HUTTER, S. LAPPAS, W. OTTO, M. KIEHNE, D. KEMENJI and F. NITZSCHE (*Monatsschrift für Brauwissenschaft,* 2003, 56, No. 11/12, 198–205).

There are many different methodologies used to detect contaminants in beer. They are all based on a pre-accumulation of organisms in/on selection media. Via micro-sieve technology a technique is presented where contaminants can be determined (without pre-accumulation) directly on a silicone sieve. Contaminants filtered onto the micro-sieve can be detected by both fluorescence and by molecular biology (PCR-analysis). As a result, statements regarding the functionality (viability) and the identity of contaminants are possible.

G.S./R.E.W.

Expression levels of the yeast alcohol acetyltransferase genes *ATF1, Lg-ATF1,* and *ATF2* control the formation of a broad range of volatile esters. K.J. VERSTREPEN, S.D.M. VAN LAERE, B.M.P. VANDERHAEGEN, G. DERDELINCKX, J.-P. DUFOUR, I.S. PRETORIUS, J. WINDERICKX, J.M. THEVELEIN and F.R. DELVAUX (*Appl. Environ. Microbiol.,* 2003, 69, 5228–5237).

During fermentation processes, yeast cells produce a broad range of aroma-active substances which greatly affect the complex flavour of fermented alcoholic beverages. While these secondary metabolites are often formed in trace amounts, their concentrations determine the distinct aroma of these beverages. Volatile aroma-active esters are responsible for the fruity character of such beverages. Esters are produced by fermenting yeast cells in an enzyme-catalyzed intracellular reaction. In order to com-

pare the roles of the known *Saccharomyces cerevisiae* alcohol acetyltransferases, *Atflp, Atf2p* and *Lg-Atflp* in volatile ester production, the respective genes were either deleted or overexpressed in a laboratory strain or a commercial brewing strain. Analysis of the fermentation products confirmed that the expression levels of *ATF1* and *ATF2* greatly affected the production of ethyl acetate and isoamyl acetate. With respect to the esters analysed in this study, *Atf2p* seemed to play only a minor role compared to *Atf1p.* Overexpression of different alleles of *ATF1* and *ATF2* led to different ester production rates, indicating that differences in the aroma profiles of yeast strains may be partially due to mutation in their *ATF* genes.

G.G.S.

Engineering redox cofactor regulation for improved pentose fermentation in *Saccharomyces cerevisiae.* R. VERHO, J. LONDESBOROUGH, M. PENTTILA and P. RICHARD (*Appl. Environ. Microbiol.,* 2003, 69, 5892–5897).

Pentose fermentation to ethanol is desired in biotechnology when fuel ethanol is to be produced from biomass. D-xylose and L-arabinose fermentation to equimolar amounts of ethanol and carbon dioxide under anaerobic conditions is theoretically possible and of relevance in biotechnology. However, in practice, ethanol fermentation requires careful aeration or the fermentation product is either biomass or xylitol and carbon dioxide. Pentose fermentation to ethanol with recombinant *Saccharomyces cerevisiae* is slow and has a low yield. A likely reason for this is that the catabolism of the pentoses D-xylose and L-arabinose through the corresponding fungal pathways creates an imbalance of redox cofactors. The process, although redox neutral, requires NADPH and NAD^+, which have been regenerated by separate processes. NADPH is normally generated through the oxidative part of the pentose phosphate pathway by the action of glucose-6-phosphate dehydrogenase. To facilitate NADPH regeneration, the recently discovered gene (GDP1), which encodes for a fungal $NADP^+$ dependent D-glyceraldehyde-3-phosphate dehydrogenase (NADP-GAPDH), has been cloned into a *S. cerevisiae* strain with the D-xylose pathway. NADPH regeneration through an NADP-GAPDH is not linked to carbon dioxide production. The resulting strain fermented D-xylose to ethanol with a higher rate and yield than the corresponding strain without GDP1. The levels of the unwanted side products xylitol and carbon dioxide were lowered. Through genetic engineering of the redox reactions, the yeast strain was converted from a strain that produced mainly xylitol and carbon dioxide from D-xylose into a strain that produced mainly ethanol under anaerobic conditions.

G.G.S.

Gene dosage effect of L-proline biosynthetic enzymes on L-proline accumulation and freeze tolerance in *Saccharomyces cerevisiae.* Y. TERAO, S. NAKAMORI and H. TAKAGI (*Appl. Environ. Microbiol.,* 2003, 69, 6527–6532).

Frozen dough technology has recently been used in the baking industry to supply oven-fresh bakery products to consumers. Many freeze-tolerant yeast strains have been isolated from natural sources and have also been constructed by conventional mutation techniques. However, the mechanism of freeze tolerance is not well understood and baker's yeast that provides good leavening qualities for both sweet- and lean-thawed doughs after frozen storage has not yet been developed. It has previously been reported that L-proline has cryoprotective activity in *Saccharomyces cerevisiae.* A freeze tolerant mutant with L-proline accumulation was recently shown to carry an allele on the *PRO1* gene encoding gamma-glutamyl kinase, which resulted in single amino acid substitution (Asp154Asn). This mutation enhanced the activities of both glutamyl kinase and glutamyl phosphate reductase, which catalyse the first two steps of proline biosynthesis. These results indicate that both enzymes are rate-limiting enzymes in yeast cells. A high tolerance for freezing correlated with higher levels of L-proline. These findings also suggest that, in addition to cryoprotective activity, intracellular L-proline could protect yeast cells from damage by oxidative stress.

G.G.S.

8) Patents issued and patent applications

The following sampling of abstracts from recently issued patents and patent applications were selected from the United States Patent and Trademark Office Website (http://www.uspto.gov/patft/) and from Europe's Network of Patent Databases (http://gb.espacenet.com). Full patent information is available at these sites (online and at no cost) if more details are desired.

Use of glycosides extracted from hop plant parts to flavour malt beverages. A.A. MURAKAMI, A. NAVARRO, D.S. RYDER and H. GOLDSTEIN, Miller Brewing Company Milwaukee, WI (*United States Patent 6,599,554, July 29, 2003*).

The isolation of at least one water soluble glycoside comprising an aromatic moiety group conjugated to mono-, di-, and trisaccharides from hop plant parts other than hop cones is disclosed. A glycoside preparation made by extraction of hop plant leaves with an aqueous alcohol was discovered to confer a pleasant grape flavor to the beer to which the glycoside preparation was added. The glycoside composition of the glycoside preparation made from aqueous alcohol-extracted hop plant leaves was found to differ from a similar preparation made from hop cones.

I.R.

Antibacterial packaging material including hop acids. M.C. BARNEY, D.S. RYDER and J.R. SEABROOKS, Miller Brewing Company Milwaukee, WI (*United States Patent Application 20,030,228,814, December 11, 2003*).

An antibacterial packaging material including a layer comprising matted cellulosic fibers is disclosed. The layer comprising matted cellulosic fibers has opposed surfaces, and a hop acid selected from alpha-acids, beta-acids, and mixtures thereof is dispersed in the fibers and between the opposed surfaces. A coating of polymeric material (e.g., a

polyolefin) may be disposed on the layer comprising matted cellulosic fibers to provide a coated material. The antibacterial packaging material is useful in that the growth of gram positive spore-forming bacteria such as Clostridium and/or Bacillus (e.g., *Bacillus anthracis*) within or on the material is inhibited. In another form, a hop acid is disposed on at least a portion of one of the surfaces of the layer comprising matted cellulosic fibers. A coating of polymeric material is disposed over the hop acid and at least over a portion of one of the surfaces of the layer comprising matted cellulosic fibers.

I.R.

Hopped malt beverage having enhanced light stability. J.R.L. BORDELEAU, D.J. HASTINGS and M.J. MCGARRITY. Labatt Brewing Company Limited London, CA (*United States Patent 6,649,204, November 18, 2003*).

An improved process for the production of a hopped malt beer, wherein a processing liquid containing riboflavin is hopped to form the desired beverage. The improvement comprises subjecting that processing liquid to an effective amount of actinic radiation of a wavelength adapted to decompose the riboflavin and thereby reduce the amount thereof, whereby a beer having enhanced light stability is obtained.

I.R.