# 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

**I. Campbell, T. Bühler, I. Russell, G.G. Stewart and R.E. Wheeler**

### 1) Raw Materials

**The Friabilimeter Network – First Study by the Network Calibration Group.** H.-M.ANGER. (*Monatsschrift für Brauwissenschaft* 55, No 3/4, 60 – 68, 2002) Use of the friabilimeter, to assist in the production-oriented, reproducible assessment of malt quality, commenced just 25 years ago, and has gained quickly in popularity since that time. In many malt delivery contracts, measurements determined by the friabilimeter have to be within close limits, which is why the results have to be obtained with the highest possible statistical reliability, to accurately reflect this aspect of raw material quality. The extent of any wear in the instruments used, is the cause, to a varying degree, of the wide distribution of the precision data gained by ring analysis. Systematic deviations occurred in ring analysis systems on the continent of Europe and in the British Isles, which increased with the course of time, and called for readjustment by users. With the production of the EBC Standard Malt 14, after the usual ring test, the friability value was set a good two percent points higher than the corresponding measurements taken with the "original friabilimeter" (the Chapon Master Friabilimeter). It became apparent that the use of the individual instruments, which were calibrated differently, quickly led to a false interpretation in the assessment of malt quality. A network was established at six European Brewing Industry Institutes, at the suggestion of the manufacturer, based on using a batch of instruments calibrated against the original Chapon Friabilimeter. Since then regular ring analyses have been carried out by this group using the EBC standard malts. This has led to the tenderness (friability) rating of the 14 standard malts being corrected by the EBC. This paper explains the foundation of the friabilimeter calibration network (FCN).

T.B./R.E.W.

**Free and Triglyceride-Bonded Hydroxy Fatty Acids in Barley and Malt. I. Influence of Variety, Region of Growth and Harvest Year** — K.WACKERBAUER and S.MEYNA, (*Monatsschrift für Brauwissenschaft* 55, No 3/4, 52 – 57, 2002) The oxidation of linoleic acid appears to be one of the most significant reaction mechanisms for the development of the first forerunner of the taste active aging carbonyls such as, for example, the well known (*E*)-2-nonenal, in beer. As a result of this it has been the usual practice for some time now in scientific brewery research to measure the free hydroxy fatty acids in all possible stages when producing beer to be able to come to possible conclusions as to the aging behaviour of the finished product. With the help of a unique method of analysis it has now been possible to obtain evidence for the first time that a considerable proportion of hydroxy fatty acids are already present in barley and malt, esterfied in the form of triacylglycerols. By means of this analytical measurement it is possible to detect eight groups of substances in oxidised linoleic acid, through the use of GC/MS: two monohydroxyoctadecane acids (HOD), two dihydroxyoctadecane acids (DHOE) and four trihydroxyoctadecane acids (THOE). The study of over twenty commercial barleys, and the malt made from them, revealed that in the majority of cases a maximum of triglyceride-bonded hydroxy fatty acids are already to be found in the barley. Moreover the average distribution of the different groups reveals approx. 50% HOD, 30% DHOE and 20% THOE in barley and malt. As far as the concentration ranges of the esterified hydroxy fatty acids to be found in the dry substance of the barley and malt are concerned, these were values from 50 ppm to 110 ppm HOD, 20 ppm to 90 ppm DHOE and 10 ppm to 65 ppm THOE. Over and above this, it was found that the variety, region of growth, and year of growth parameters have a considerable influence on the quantity of this possible aging taste forerunner.

T.B./R.E.W.

### 2) Beer—Analysis

**The Kübler Bierdest 2 for Fast, Distillation Based Determination of Significant Quality Parameters for Beer.** P. SCHROPP and F. MÖSEL, ( *Monatsschrift für Brauwissenschaft* 55, No 3/4, 70 – 74, 2002) Distillation analysis is valid now as before as the reference method to determine the original gravity, alcohol, extract and degree of fermentation of beer. The Kübler Bierdest 2 simplifies and shortens this method by using water vapour distillation. Its performance in respect of repeatability and accuracy has been carefully studied. The findings reveal a repeatability that is almost identical with that of standard distillation. The accuracy is within a very close tolerance range of max. ± 0.1% in the case of original gravity and alcohol compared with the standard distillation methods. The possibility of photometric determination of vicinal

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alcohol compared with the standard distillation methods. The possibility of photometric determination of vicinal diketones, using this apparatus, extends the area of application in brewery laboratories.

T.B./R.E.W.

### 3) Brewing—Fermentation

**Brewing. The Evolution of a Tradition into a Technology**. G. G. STEWART (*Ingenia*, February 2002, 31-35). This review of brewing in the Journal of the Royal Academy of Engineering discusses the maintenance of yeast quality for re-pitching, maturation and biological, chemical and head stability of beer. The technologies of high gravity brewing and continuous fermentation and maturation by free or immobilised cells are also described in detail.

I.C.

### 4) Microbiology

**Interaction of SMKT, a Killer Toxin Produced by *Pichia farinosa*, with the Yeast Cell Membrane.** C. SUZUKI, Y. ANDO and S. MACHIDA (*Yeast*, 2001, **18**, 1471-1478) Salt-mediated killer toxin (SMKT) produced by the halotolerant yeast *P. farinosa* in the presence of high concentrations of NaCl or sorbitol, kills yeasts of various genera, including *Saccharomyces*. A sensitive strain of *S. cerevisiae* was used to investigate the interaction with SMKT. Cells were treated with 5☒M SMKT for 5 min, washed free of unbound SMKT and then treated with sodium carbonate to extract protein associated with membranes. Both ❣α and ♭β sub-units of the toxin molecule were extracted by sodium carbonate, but since 3-phosphoglycerate kinase (used as a marker of cytoplasmic protein) was not detected, neither sodium carbonate nor SMKT caused cell lysis. The role of the cell wall was investigated by comparing the effect of SMKT on intact cells and spheroplasts. Viability of intact cells fell to 3.6% after 1 h treatment; regeneration of protoplasts was reduced from 28% in untreated cells to 11.4% after 1 h SMKT treatment. SMKT was incorporated in liposomes at pH 4.5 to investigate the interaction between the pH-sensitive killer toxin and cell membranes. The biochemistry was not elucidated but microscopic examination of SMKT-treated cells confirmed the lysis of both intact cells and spheroplasts.

I.C.

**Glycogen, a Covalently Linked Component of the Cell Wall in *Saccharomyces cerevisiae*.** A. U. ARVINDEKAR and N. B. PATIL (*Yeast*, 2002, **19**, 131-139). Glycogen occurs in *S. cerevisiae* both intracellularly, as a soluble reserve carbohydrate, and in the cell wall, rendered water-insoluble by its covalent linkage to ♭ 1-6 branches of the ♭-glucan. The wall-bound glucan was solubilised by ♭ 1-3 glucanase and isolated free from ♭-glucan by affinity chromatography. Alternatively, water-insoluble glucan could be extracted by acetic acid. Therefore some of the anomalies of previous research on yeast glycogen can be explained by release of unsuspected wall-bound material.

I.C.

**A History of Research on Yeasts 4: Cytology part 1, 1890 – 1930**. J. A. BARNETT and C. F. ROBINOW (*Yeast*, 2002, **19**, 151-182) The contribution of cytological studies to early research on yeasts is comprehensively reviewed, e.g. the initial confusion between nucleus and vacuole, the genetic function of chromosomes, and modes of sporulation.

I.C.

**Genetically Controlled Self-Aggregation of Cell-Surface-Engineered Yeast Responding to Glucose Concentration.** W. ZOU, M. UEDA and A. TANAKA. (*Applied and Environmental Microbiology,* 2001, **67**, 2083-2087) In brewing, fermentation and some other bioprocesses, separation of cells from broth is essential. Centrifugation of the broth is commonly used to achieve complete separation, but its capital costs and operation can be complicated and expensive. Flocculation and agglutination in *Saccharomyces cerevisiae* is used in brewing and in the production of foods and medical substances. A cell-surface-engineered yeast has been constructed displaying two types of agglutinin (modified a-agglutinin and α-agglutinin) as the cell surface, with agglutination being independent of both mating type as pheromes. The modified a-agglutinin could induce agglutination of cells displaying Agα1p (α-agglutinin). This modified yeast, displaying both agglutinins, agglutinated and sedimented in response to decreased glucose concentration. When the glucose concentration was high, this modified yeast grew normally. In late logarithmic phase, when the glucose concentration became very low, agglutination occurred suddenly and yeast cells sedimented completely. Strains, in which aggregation can be genetically controlled, can be used in industrial processes in which the separation of yeast cells from the supernatant is necessary.

G.G.S.

**Physiological Properties of *Saccharomyces cerevisiae* from which Hexokinase II Has Been Deleted.** J.A. DIDERICH, L.M. RAAMSDONK, A.L. KRUCKEBERG, J.A. BERDEN and K. VAN DAM. (*Applied and Environmental Microbiology,* 2001, **67**, 1587-1593) Glycolysis plays a central role in glucose metabolism in *Saccharomyces cerevisiae*. It is the root for many different pathway branches which lead primarily to the production of biomass, ethanol and $CO_2$. The first step in glycolysis is the transport of glucose across the cell membrane by members of the hexose transporter family. Subsequently, intracellular glucose is phosphorylated to glucose-6-phosphate. There are three isozymes that phosphorylate glucose: glucokinase (encoded by *GLKI*), hexokinase I (encoded by *HXKI*), and hexokinase II (encoded by *HXK2*). These isozymes have different affinities for glucose and ATP and different specificities toward other sugars, such as fructose and mannose. Hexokinase II is an enzyme central to glucose metabolism and repression. Deletion of HXK2 dramatically altered the physiology of *S. cerevisiae*. The mutant strain displayed full oxidative growth at high glucose concentrations in early exponential batch culture, resulting in an initial absence of fermentative products such as ethanol, a postponed and shortened diauxic shift, and high biomass yields. Several intracellular changes were associated with the deletion of hexokinase II. The *hxk2* mutant had higher mitochondrial

$H^+$-ATPase activity and lower pyruvate decarboxylase activity, which coincided with intracellular accumulation of pyruvate in the *hxk2* mutant. The results suggest a redirection of carbon flux in the *hxk2* mutant in the production of biomass as a consequence of reduced glucose repression.

G.G.S.

**Yeast Population Dynamics During the Fermentation and Biological Aging of Sherry Wines.** B. ESTEVE, M.J. PERIS-TORAN, E. GARCIA-MAIQUEZ, E. URUBURN and A. QUEROL. (*Applied and Environmental Microbiology,* 2001, **67**, 2056-2061) The production of sherry wines comprises two successive processes: first, alcoholic fermentation of must by yeast to produce white wine, and second, biological aging of the wine under a velum ("flor") produced by yeast, the so-called flor yeast. All wines made by this special procedure (including finos, amotillodos and olorosos) are called sherry wines. Molecular and physiological analyses were used to study the evolution of the yeast population from alcoholic fermentation to biological aging. The four races of "flor" *Saccharomyces cerevisiae* (*beticus, cheresiensis, mortuliensis* and *rouxii*) exhibited identical restriction patterns for the region spanning the internal transcribed spacers 1 and 2 and the 5.85 rRNA gene, but this pattern was different from those exhibited by non-flor *S. cerevisiae* strains. This flor-specific pattern was detected only after wines were fortified, never during alcoholic fermentation, and all the strains isolated from the velum exhibited the typical flor yeast pattern. By restriction fragment length polymorphisim of mitochondrial DNA and karyotyping, it has been demonstrated that: (i) the native strain is better adapted to fermentation conditions than commercial strains; (ii) two different populations of *S. cerevisiae* strains are involved in the process of elaboration of fine sherry wine, one of which is responsible for must fermentation and the other, for wine aging, and (iii) one strain was dominant in the flor population integrating the velum for sherry wines produced in Gonzalez Byass wines. It is concluded that yeast population dynamics during biological aging is a complex phenomenon and differences between yeast populations from various wineries can be observed.

G.G.S.

**Auxotrophic Yeast Strains in Fundamental and Applied Research.** J.T. PRONK. (*Applied and Environmental Microbiology,* 2002, **68**, 2095-2100) Molecular genetic tools have become increasingly important in fundamental and applied yeast research. Genetic modifications, such as the targeted inactivation of genes or their controlled (usually over) expression from episomal or integrating vectors, require the use of selectable marked genes for efficient detection and selection of transformed cells. Auxotrophic yeast strains continue to be convenient platforms for applied research, especially in the field of heterologous gene expression. When such strains are used for reasons of practicality or economy, comparison of strains with nonmatching auxotrophies should be avoided and auxotrophically required nutrients should be provided in amounts that prevent them from becoming growth limiting. Even when prototrophic strains are used, great care should be exercised in the design of synthetic growth media in order to avoid "hidden" nutrient limitations. Since nutritional requirements of microorganisms vary as a function of growth conditions and genetic background, it is essential to experimentally verify which nutrient limits biomass formation even when prototrophic strains are used. The physiological properties of different *Saccharomyces cerevisiae* strains can differ greatly. At a time when public domain databases have become vital tools for studies on popular model organisms such as *S. cerevisiae*, it is important to minimize the confusion that may arise from comparison of quantitative data from different genetic backgrounds. It is therefore worthwhile to strive for a limited number of well-defined and experimentally accessible reference strains. Based on a comparison of four candidate strains, a consortium of European yeast research groups has recently proposed prototrophic strains of the CEN.PK family of *S. cerevisiae* as a useful platform for integrated genetic and physiological studies on this yeast.

G.G.S.

**Reduced Oxidative Pentose Phosphate Pathway Flux in Recombinant Xylose-Utilizing *Saccharomyces cerevisiae* Strains Improves the Ethanol Yield of Xylose.** M. JEPPSSON, B. JOHANSSON, B. HAHN-HAGERDAL and M.R. SORWA-SRAUSLUND. (*Applied and Environmental Microbiology,* 2002, **68**, 1604-1609) Fuel ethanol production from fermentation of lignocellulosic hydrolysates is an attractive replacement for liquid fossil fuels because its production is renewable and it does not generate net carbon dioxide. Hydrolysis of lignocellulose generates mostly hexose but also some pentose sugars. The pentose sugars cannot be metabolised by *Saccharomyces cerevisiae*, however xylose must be fermented to ethanol for the process to be economically feasible. The yeast *Pichia stipitis* metabolizes xylose through expression of the *XYL1* gene, encoding xylose reductase (XR), and the *XYL2* gene, encoding xylitol dehydrogenase (XDH). Unfortunately, *P. stipitis* is sensitive to ethanol and requires low and carefully controlled oxygenation which precludes its use for industrial ethanol production. Recombinant *S. cerevisiae* strains expressing the *XYL1* and *XYL2* genes from *P. stipitus* have been constructed and can ferment xylose. Xylitol production can be lowered by overexpression of the XKSI gene, which encodes for the native xylulokinase (XK), but still approximately one third of the xylose is converted to xylitol under anaerobic conditions. Xylitol formation can result from the cofactor imbalance between the NADPH-consuming XR and the NADH-producing XDH reactions. The ethanol yield has been increased and xylitol yield decreased by lowering the flux through the NADPH-producing pentose phosphate pathway. These results indicate that xylitol production is strongly connected to the flux through the oxidative part of the pentose phosphate pathway.

G.G.S.

**Influence of Medium Buffering Capacity on Inhibition of *Saccharomyces cerevisiae* Growth by Acetic and**

**Lactic Acids.** K.C. THOMAS, S.H. HYNES, and W.M. INGLEDEW. (*Applied and Environmental Microbiology,* 2002, **68**, 1616-1623) *Saccharomyces cerevisiae* under aerobic conditions can use short-chain organic acids, such as acetic and lactic acids, as carbon sources. The process involves induction of certain anabolic pathways, enzymes and specific transport mechanisms. If glucose is available in the growth medium, these pathways and permeases are repressed. Acetic acid (167mM) and lactic acid (548 mM) completely inhibited growth of *S. cerevisiae* both in minimal medium and in media which contained supplements, such as yeast extract, corn steep powder, or a mixture of amino acids. However, the yeast grew when the pH of the medium containing acetic acid or lactic acid was adjusted to 4.5, even though the medium still contained the undissociated form of either acid at a concentration of 102 mM. The results indicated that the buffer pair formed when the pH was adjusted to 4.5 stabilized the pH of the medium by sequestering protons and by lessening the negative impact of the pH drop on yeast growth, and it also decreased the difference between the extracellular and intracellular pH values ($\triangle$pH), the driving force for the intracellular accumulation of acid. Increasing the undissociated acetic acid concentration at pH 4.5 to 163 mM by raising the concentration of the total acid to 267 mM did not increase inhibition. It is suggested that this may be the direct result of decreased acidification of the cytosol because of the intracellular buffering by the buffer pair formed from the acid already accumulated. At a concentration of 102 mM undissociated acetic acid, the yeast grew to higher cell density at pH 3.0 than at pH 4.5, suggesting that it is the total concentration of acetic acid (104 mM at pH 3.0 and 167 mM at pH 4.5) that determines the extent of growth inhibition, not the concentration of undissociated acid alone.

G.G.S.

### 5) Processing—Automation

**Automated Production Support for the Bioprocess Industry.** B. LENNOX, K. KIPLINE, J. GLASSEY, G. MONTAGUE, M. WILLIS and H. HIDEN. (*Biotechnology Progress,* 2002, **18**, 269-275) Production facilities in the process industries are under continuous demands to improve their efficiency of operation and the quality and consistency of their product. This represents a real and difficult challenge to systems engineers. The complexity of the problem comes as a result of the many generic features of process plants, a selection of which are: production facilities are becoming increasingly complex and consequently more difficult to fully understand; the quality of information obtained from the process (by way of on-line sensors and off-line laboratory analysis) is often limited; there are many problems that may adversely affect the operation of the process; there are often multiple responses that can be made to rectify any given problem, some of which will provide better results than others; there can be large delays before the effects of a change in the process are observed, thus often making it difficult to ensure that future production meets quality requirements. This paper describes the application of Artificial Intelligence and Multivariate Statistical Techniques to two industrial fermentation systems. In the first example, an Expert System is shown to provide tighter control of an important process parameter. This is shown to lead to improved consistency of operation. In the second application, Principal Component Analysis is applied to a final stage fermentation production facility. The results presented indicate that the algorithm can provide concise indicators of process faults that can be presented to the operators to assist them in taking suitable corrective actions.

G.G.S.

### 6) Processing—Filtration

**Significance of Deep Filtration for Kieselguhr Filtration of Beer (1)**. K. HUSEMANN, F. HEBMÜLLER and M. EBLINGER. (*Monatsschrift für Brauwissenschaft* 55, No 3/4, 44 – 50, 2002) Mechanical processing technology differentiates filtration processes according to their working principles by surface and deep filtration. Cake filtration is the process which in the case of the filter cake takes over the job of the filter medium in the course of the process and is classified as surface filtration, and as such the usual "models" have been studied. In this case it is felt that both working principles can be superimposed. The kieselguhr filtration of beer is considered to be deep filtration, although the extent of deep filtration is given little consideration in the literature. Deep filtration generally does not play any role when determining filtration processes. In a joint project of the Technical University Mining Academy Freiberg and Freiberger Brauhaus AG the significance of deep filtration for kieselguhr filtration of beer has been examined more closely.

T.B./R.E.W.

### 7) Patents Issued and Patent Applications

**Zero Oxygen Permeation Plastic Bottle for Beer and Other Applications.** P.J.CAHILL et al, BP CORPORATION NORTH AMERICA INC. (CHICAGO, IL) *(United States Patent 6,365,247, April 2002)* Multilayered plastic bottles are disclosed having oxygen scavenging capacity sufficient to maintain substantially zero or near zero (depending on product requirements) presence of oxygen in the bottle cavity for the planned shelf life of the bottled product under specified storage conditions. The bottles feature a layer comprised of oxygen scavenger copolyester and may be used for bottling beer and other products requiring nearly total absence of oxygen for the duration of the target product shelf life.

I.R.

**Compositions for Producing Fermented Malt Beverages.** R.RANGEL-ALDAO, A.BRAVO, B.SANCHEZ, I.GALINDO-CASTRO, CERVECERIA POLAR, CA (CARACAS, VE) *(United States Patent 6,372,269, April 2002)* The present invention is directed to a method for stabilizing the flavour of a fermented malt beverage**,** most particularly a beer, by the addition of one or more inhibitors, blockers, reducing agents or binding agents that inactivate one or more Maillard reaction intermediates that induce staling of the flavour of fermented malt beverages. In preferred such methods, the agents used are reductase enzymes, especially aldehyde reductases, carbonyl reductases, aldose reductases, oxoaldehyde reductases and most particularly oxidoreductases produced by yeasts such as

isozymes of Old Yellow Enzyme (e.g., OYE1, OYE2 and OYE3). The invention is also directed to the fermented malt beverage prepared by such a method, and to the use during the brewing process of reductase enzymes from naturally occurring sources, including those produced by yeasts, to stabilize the flavour of the resulting beer product and to produce a beer having a stable flavour. The invention also relates to cells which have been specifically modified, selected, or genetically engineered to express or secrete a reductase enzyme which may be used during the brewing process to stabilize the flavour of the resulting beer product and to produce a beer having a stable flavour, and to compositions comprising such cells or recombinant enzymes. The invention also provides fermented malt beverages having enhanced flavour stability produced by these methods.

I.R.

**Process for Producing an Extract of an Accelerated Oak Aged Alcoholic Concentrate.** J.A. ZIMLICH III, BROWN-FORMAN CORPORATION (LOUISVILLE, KY) *(United States Patent 6,344,226, February 2002)* An extract of an oak aged alcoholic product is produced by adding a food grade solvent, such as ethyl acetate, to the oak aged alcoholic product and mixing the two liquids. The resulting mixture is allowed to separate into two layers, a first layer and a second layer. The first layer, including the food grade solvent, flavours, colour, alcohol (i.e., ethanol) and water, is separated from the second layer. The solvent is then removed from the first layer to produce the extract. The extract can be added to a less costly alcoholic beverage, such as grain neutral spirits, or sugar beet spirits, to produce a beverage having the taste of a mature oak aged alcoholic beverage. Also disclosed is an accelerated whisky maturation method, including combining an alcoholic distillate with toasted oak chips, heating and aerating and/or oxygenating the resulting mixture to produce an accelerated oak aged alcoholic product. The toasted oak chips are then removed.

I.R.