

## 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

**Genetically Modified Foods: Threat or Opportunity?** S. ROLLER. (*Food Technology & Biotechnology*, 2001, **39**, 259-263) It has been estimated that about 800 million people worldwide have eaten genetically modified (GM) foods or food ingredients without adverse consequences on their health. There have been no reports of deaths caused by the consumption of GM foods. Gene technology has the potential to offer many improvements in the quality and quantity of the world's food supply provided that genuine concerns regarding safety, environmental impact, information and ethics are satisfactorily addressed. In this paper, some of the benefits as well as concerns about genetically modified foods are discussed using examples such as tomatoes, soybeans, corn (maize) or rice.

G.G.S.

**Genetically Modified Barley and the Brewing Industry.** G.J. MUEHLBAUER and D.A. SOMERS. (*Technical Quarterly of the Master Brewers Association of the Americas*, 2001, **38**, 145-154) The term "genetically modified," and its abbreviation "GM" is frequently used to describe organisms that have been genetically engineered. This review does not refer to plant improvement through traditional means of identifying variation or breeding. Genetic engineering is a biotechnological development for transferring genes from one organism to any other organism. All crops used in the production of beer, with the exception of hops, can be genetically modified. Currently, barley is being genetically modified for research purposes and will be commercialised in the near future whilst other GM crops such as maize (corn) and rice are in commercial production. This review considers a number of specific topics including: (i) the history and status of plant genetic engineering technologies with focus on barley and other cereals, (ii) GM crops and traits currently in production and those being developed for future commercialisation, (iii) risks, and benefits of GM crops including barley and crops used a brewing ingredients, and (iv) methods for the detection of GM ingredients.

G.G.S.

**Beta-Glucan Content in Caryopses, Malt and Wort of the Selected Spring Barley Varieties.** V. PSOTA, J. EHRENBERGEROVÁ, P. HAVLOVÁ and J. HARTMANN. (*Monatsschrift für Brauwissenschaft*, 2002, **55**, No 1/2, 10-14, 27) Beta-glucan content in caryopses (barley grains), malt and wort was observed for 10 varieties of Czech, Slovak and German origin. Significant inter-variety differences were present. The impact of the growing site also played an important role, however the impact of the year was irrelevant. At the same time the relationships between beta-glucan content and further technological features were examined. Beta-glucan content in barley caryopsis (grain) was influenced by the weight of a thousand kernels (TKW) and further by the protein content in the caryopsis (grain). The content of beta-glucan in the wort was influenced by the beta-glucanase index, the friability of the malt and the Kolbach value. The method of stepwise Multiple Linear Regression (Stepwise Selection) was used to choose the parameters significantly influencing beta-glucan content.

T. B./R.E.W.

#### 2) Brewing—Fermentation

**A Primer on Yeast Propagation Technique and Procedures.** J. EDGERTON. (*Technical Quarterly of the Master Brewers Association of the Americas*, 2001, **38**, 167-174) Yeast used to have a mystique with brewers. Today, modern philosophies take a more scientific view. Yeast is a genetically complex biological entity and consequently is subject to outside stresses and other influences that can damage it and adversely affect its performance. Mutation, selection and contamination can make a culture undesirable for use in a brewery. Introducing a new yeast culture into a brewery is a relatively straightforward procedure and can be achieved with careful technique and minimal equipment. According to the author, cultures are usually stored on agar slopes or freeze dried. The best way to begin the propagation procedure is to use the single-cell culture technique and select the "best looking" colonies for a production culture. Judicious single culture selection helps to ensure the genetic purity of the initial production culture and permits exclusion of wild yeast, respiratory deficient mutants and bacteria. Following selection of the pure culture, the yeast quantity is gradually built up, first in the laboratory and then in the pilot plant and/or brewery. When sufficient quantity of yeast is

achieved it is pitched to production. The culture is analysed by traditional methods for performance and health.

G.G.S.

**Does Osmotic Pressure affect Yeast Performance in High Gravity Fermentation?** J. HAMMOND, D. DAVIS, M. LEE and K. STOREY (*Proceedings of the European Brewery Convention, Budapest, 2001, 316-325*) It has often been claimed that the inhibitory effects of high gravity fermentations on yeast are both the osmotic stress of the high initial sugar concentration and the toxicity of the ethanol product. To test the inhibitory effects of sugar concentration, *Saccharomyces cerevisiae* NCYC1324 (lager) and NCYC 1681 (ale) were grown in wort or synthetic medium containing up to 25% (w/v) maltose. Fermentation profiles in 10% to 25% maltose showed identical rates of fall of SG. Biomass, ethanol and glycerol production showed little difference over the first 70 h of fermentation, so such divergence as occurred later could not be related to the initial conditions. Stressed yeasts would have been expected to produce larger quantities of glycerol, a known osmo-protectant. Also, ethanol or sorbitol were added to actively fermenting cultures of NCYC 1324 and the effect on CO<sub>2</sub> evolution was measured. Sorbitol, a non-metabolisable sugar, was added hourly over 8 h to a total of 32% to increase osmotic pressure of the culture medium: although CO<sub>2</sub> production fell slightly with the earliest additions it was quickly restored to normal. On the other hand, successive hourly additions of ethanol to 16% (v/v) reduced CO<sub>2</sub> production to zero. This effect was specifically due to ethanol: higher alcohols, ethyl acetate and acetaldehyde had no inhibitory effect. Therefore it is concluded that the inhibitory effects of high-gravity fermentations are caused entirely by ethanol concentration.

I.C.

**Fermentation of High Gravity Worts—Its Influence on Yeast Metabolism and Morphology.** G.G. STEWART (*Proceedings of the European Brewery Convention, Budapest, 2001, 344-352*) There are definite benefits from high gravity (HG) brewing but this paper concentrates on three of the problems: excessive ester formation, loss of foam stability and physiological damage to the yeast. Ester formation is affected by, among other factors, the range of fermentable sugars in the wort. The HG worts were prepared with standard maltose syrup (MS, 55% maltose, 15% glucose) and very high MS (VHMS, 70% maltose, 5% glucose). In experimental fermentations, of course the 12°P wort produced the lowest ester levels (reported as ethyl acetate) but of the 20°P worts, the standard (55%) MS almost doubled ester levels throughout the fermentation whereas with VHMS the increase was only approx. 30%. Similarly, 23% higher iso-amyl acetate was produced by standard MS compared with the VHMS. Also, with both high and normal gravity worts, higher maltose levels produced yeasts of higher viability and vitality. The poorer head stability of HG beers can be attributed to the proportionately greater loss of hydrophobic polypeptides (HPP) in HG brewing. On dilution of a HG beer to its normal sales strength of 4.5% abv, the level of HPP was less than half that of the standard gravity beer, and the poorer head

retention was obvious by eye. Compared with the standard 10°P wort, the level of HPP was only 25% higher at 20°P (i.e., well below the double quantity that the principle of HG brewing would require). HPP was approx. 15% lower by the end of fermentation, and after dilution to sales gravity was only about 30% of the level in the standard beer. The increased loss of HPP could be explained by the doubled rate of excretion of protease A during HG fermentation. Also, physiological damage to the yeast was demonstrated by increased wrinkling of the cell surface, reduced cell volume and aberrant vacuolar morphology in HG fermentations.

I.C.

### 3) Microbiology

**Microbiological Media for Bacteria and Wild Yeast Detection in the Brewery.** G. SPEDDING and T.P. LYONS. (*Brewers Digest, 2001, July/August, 66-70.*) The ideal situation for the lager and ale brewer is to have only one species of organism present in the wort and beer. However, because of the abundance of microorganisms present in the environment, the ideal situation is never achieved. There is an abundance of growth media available to the brewery microbiologist. The choice of which media to employ (brewing yeast, wild yeast, mould or bacteria for detection and/or identification) is not an easy one. This publication provides a review of the properties of selected media appropriate for the brewing microbiologist. The subject is handled from the perspective of a microbiologist in a craft brewery. The selection and use of such media are described. In addition, the properties of a variety of brewing microbial contaminants are discussed.

G.G.S.

**Evolution of the Lactic Acid Bacterial Community During Malt Whisky Fermentation: A Polyphasic Study.** S. van BEEK and FERGUS G. PRIEST. (*Applied and Environmental Microbiology, 2002, 68, 297-305*) In Scotch malt whisky production the wort is not boiled in order to retain the activity of the soluble enzymes from the malt during the fermentation and to maximize alcohol yield. Consequently, bacteria from the malt that can survive mashing and enter the fermentation, resulting in a mixed yeast-bacterial fermentation. If large numbers of lactobacilli enter the fermentation they compete for nutrients with yeast cells and reduce the ethanol yield. The development of the lactic acid bacterial community in a commercial malt whisky fermentation occurred in three broad phases. Initially, bacteria were inhibited by strong yeast growth. In its early stage, both cocci and rods that were partly derived from the wort and yeast were identified but some stemmed from the distillery. The middle phase began 35–40 h after yeast inoculation and was characterised by exponential growth of lactobacillus and residual yeast metabolism. *Lactobacillus casei* or *Lactobacillus paracasei*, *Lactobacillus fermentum* and *Lactobacillus ferintoshensis* were detected in samples of fermenting wort at this stage. Bacterial growth was accompanied by the accumulation of acetic and lactic acids and the metabolism of residual maltoligosaccharides. By 70 h, bacteria were present that

were phylogenetically related to *Lactobacillus acidophilus* and *Lactobacillus delbrueckii* and strains similar to the former had previously been recovered from Japanese malt whisky fermentations. These were probably obligately homofermentative bacteria that required malt wort for growth and could not be cultured on normal laboratory media, such as MRS media. The possibility that flavour could be modified by careful attention to the balance of these various bacteria present is discussed.

G.G.S.

**Acetic Acid and Lactic Acid Inhibition of Growth of *Saccharomyces cerevisiae* by Different Mechanisms.** N.V. NARENDRANATH, K.C. THOMAS and W.M. INGLEDEW. (*Journal of the American Society of Brewing Chemists*, 2001, **59**, 187-197) Alcohol produced in distilleries is not all consumed as liquor. It is also utilized for pharmaceuticals, for vinegar production, as an industrial chemical and for fuel. Some of this alcohol is not produced with the same vigorous control of bacterial contamination that is common to breweries. As a result, yields are reduced because of diversion of substrates away from alcohol to weak acids such as acetic and lactic. Stuck or sluggish fermentations can result. In this study two strains of *Saccharomyces cerevisiae* were grown in minimal media with glucose as the carbon source with added acetic or lactic acid. The intracellular pH (pHi) of yeast cells was not significantly affected by acetic acid to a maximum concentration of 0.25% (w/v). The pHi was maintained at a more or less constant value through increased activity of plasma membrane H<sup>+</sup>-adenosine triphosphatase (H<sup>+</sup>-ATPase), which pumps protons out of the cell. With lactic acid at a concentration of 0.4% (w/v) or higher, the pHi decreased sufficiently to influence yeast growth. This pHi decrease was the result of reduced membrane-bound H<sup>+</sup>-ATPase activity. Cell membrane lipids of yeast cells grown in the presence of 0.5% (w/v) lactic acid contained considerably reduced levels of the two unsaturated fatty acids palmitoleic and oleic. With acetic acid the changes in fatty acid composition were much smaller. These results indicate that acetic and lactic acids inhibit the growth of *S. cerevisiae* by different mechanisms.

G.G.S.

**Decrease in Cell Surface Galactose Residues of *Schizosaccharomyces pombe* Enhances its Coflocculation with *Pediococcus damnosus*.** X. PENG, J. SUN, C. MICHIELS, D. ISERENTANT and H. VERACHTERT. (*Applied and Environmental Microbiology*, 2001, **67**, 3413-3417) Yeast flocculation is the spontaneous aggregation of cells to form clumps that can be easily separated from the medium and plays an important role in the brewing industry. In most cases, cell wall glycoproteins are involved and induce flocculation by binding lectin type sugar receptors on neighbouring cells. *Pediococcus damnosus* can coflocculate with *Saccharomyces cerevisiae* and cause beer acidification that may or may not be desired. Similar coflocculations occur with other yeast except for *Schizosaccharomyces pombe* which has galactose-rich cell walls. The coflocculation rates of *S. pombe* wild type species with a mannose-to-galactose ratio of 5:6 in the cell wall were compared to glycosynthetic mutants that pos-

sessed a cell wall mannose-to-galactose of 5:1. These mutants coflocculated at a much higher level (30–45%) than that of its wild type (5%). Coflocculation of the mutants was inhibited by exogenous mannose but not by galactose. It is concluded that mannose residues on the cell surface of *S. pombe* serve as receptors for a *P. damnosus* lectin but that these receptors are shielded by galactose residues in wild-type strains. Such interactions are important in the production of Belgian acid types of beers in which mixed cultures are used to improve flavour.

G.G.S.

**Novel Pathway for Alcoholic Fermentation of  $\delta$ -Gluconolactone in Yeast *Saccharomyces bulderi*.** J. P. van DIJKEN, A. van TUIJL, M.A.H. LUTTIK, W.J. MIDDECHOVEN and J.T. PRONK. (*Journal of Bacteriology*, 2002, **84**, 672-678) In the standard tests employed for the identification and classification of yeasts, the ability to perform alcoholic fermentation is only tested with sugars and sugar oligomers. Hitherto, substrates with different degrees of reduction have been assumed to be nonfermentable by yeasts. Recently this assumption has been disproved because it has been shown that  $\delta$ -gluconolactone could be fermented to ethanol and CO<sub>2</sub> by a *Saccharomyces bulderi* strain isolated from corn silage. Under anaerobic conditions, *S. bulderi* rapidly ferments this sugar to ethanol and CO<sub>2</sub>. A novel pathway for  $\delta$ -gluconolactone fermentation operates in this yeast. In this yeast  $\delta$ -gluconolactone is first reduced to glucose via an NADPH-dependent glucose dehydrogenase. After phosphorylation, half of the glucose is metabolised via the pentose phosphate pathway yielding the NADPH required for the glucose-dehydrogenase reaction. The remaining half of the glucose is dissimilated via glycolysis. The ability of *S. bulderi* to use the pentose phosphate pathways as an efficient, high-throughput catabolic pathway, under strict anaerobic conditions, may be an important advantage for efficient xylose fermentation by engineered strains.

G.G.S.

**Characterisation of *Schizosaccharomyces pombe* Malate Permease by Expression in *Saccharomyces cerevisiae*.** C. CAMARASA, F. BIDARD, M. BONY, P. BARRE and S. DEQUIN. (*Applied and Environmental Microbiology*, 2001, **67**, 4144-4151) Elimination of the L-malic acid present in grapes is of considerable technological value in wine making because it results in the deacidification and stabilization of the wine. This substrate is traditionally eliminated by lactic acid bacteria which carry out malolactic fermentation after alcoholic fermentation. However, L-malic acid degradation is uncertain, as the poor growth of lactic acid bacteria at low pH often delays malolactic fermentation and may even prevent it altogether. Malate utilization of *Saccharomyces cerevisiae* is limited by the lack of a malate transporter. This is not the case with *Schizosaccharomyces pombe* which contains a malate permease (*MAE1*) which has recently been cloned into *S. cerevisiae* resulting in a marked increase in L-malate utilization. This study has provided insight, in physiological and kinetic terms, into the way in which this permease is encoded by the *MAE1* gene function. In particular, it has been established that the *Mae1p* permease transported the

monoanionic form of L-malate, as a function of the transmembrane substrate and pH gradients.

G.G.S.

**Xylulokinase Overexpression in Two Strains of *Saccharomyces cerevisiae*, also Expressing Xylose Reductase and Xylitol Dehydrogenase and Its Effect on Fermentation of Xylose and Lignocellulosic Hydrolysate.** B. JOHANSSON, CH. CHRISTENSSON, T. HOBLEY and B. HAHN-HAGERDAL. (*Applied and Environmental Microbiology*, 2001, **67**, 4249-4255) A yeast strain capable of fermenting xylose and glucose to ethanol with high yields would increase the economic feasibility of fuel ethanol production from lignocellulosic biomass. *Saccharomyces cerevisiae* cannot ferment xylose but can ferment its isomer, xylulose. In yeast, xylose reductase (*XR*) and xylitol dehydrogenase (*XDH*) catalyze the conversion of xylose to xylulose via the intermediate xylitol. The gene (*XKSI*) encodes the xylulose-phosphorylating enzyme xylulokinase. The effect of *XRS1* overexpression on two different *S. cerevisiae* host strains has been studied. Fermentations were carried out in defined and complex media containing a hexose and pentose sugar mixture on a birch wood lignocellulosic hydrolysate. *XKSI* overexpression increased the ethanol yield by a factor of 2 and reduced the xylitol yield by 70–100% and the final acetate concentrations by 50–100% in one of the yeast strains studied, and in the other reduced the total xylose consumption by half. Yeast extract and peptone partly restored sugar consumption in the hydrolysate medium. The results demonstrate that strain background and modulation of *XKSI* expression are important for generating an efficient xylose-fermenting recombinant strain of *S. cerevisiae*.

G.G.S.

**Generation of a Novel *Saccharomyces cerevisiae* Strain that Exhibits Strong Maltose Utilization and Hyperosmotic Resistance using Nonrecombinant Techniques.** V. HIGGINS, P.J.L. BELL, I.W. DAWES and P.V. ATT-FIELD. (*Applied and Environmental Microbiology*, 2001, **67**, 4346-4348) Two categories of *Saccharomyces cerevisiae* are used in the modern baking industry. These are yeast strains optimised for use in dough containing no added sugar (unsugared dough) and yeasts that are specialized for use in sweet dough to which sugar has been added. A yeast strain capable of leavening both unsugared and sweet bread dough efficiently would reduce the necessity of producing multiple baker's yeast strains. However, issues involving the use of genetically modified foods have rendered the use of recombinant techniques for developing yeast strains controversial. This paper describes the use of selection and screening systems, in conjunction with traditional mass mating techniques to develop a baker's yeast strain that effectively leavened both types of dough.

G.G.S.

**Improved Properties of Baker's Yeast Mutants Resistant to 2-Deoxy-D-glucose.** A.M. RINCON, A.C. CODON, F. CASTREJON and T. BENITEZ. (*Applied and Environmental Microbiology*, 2001, **67**, 4279-4285) *Sac-*

*charomyces cerevisiae* can utilize a variety of carbon sources, but glucose and fructose are preferred. When one of these sugars is present, carbon catabolite repression occurs and the enzymes required for utilization of alternative carbon sources are synthesized at low rates or not at all. Spontaneous mutants from a baking strain of *S. cerevisiae* resistant to 2-deoxy-D-glucose exhibited improved fermentative capacity on sweet doughs. Three mutants grew at the same rate as the wild type in minimal defined medium. Two of the mutants also had high levels of phosphatase active on 2-deoxy-D-glucose-6-phosphate. Dough fermentation (CO<sub>2</sub> liberation) by two of the mutants was faster and/or produced higher final bread volumes than by the wild type both under laboratory and industrial conditions, when the doughs were supplemented with glucose or sucrose. However, the three mutants were slower when fermenting plain doughs. Fermented sweet bakery products obtained with these mutants were of better quality than those produced by the wild type, with regard to their texture and organoleptic properties.

G.G.S.

**Control of Higher Alcohol Production by Manipulation of the *BAP2* Gene in Brewing Yeast.** Y. KODAMA, F. OMURA, K. MIYAJIMA and R. ASHIKARI. (*Journal of the American Society of Brewing Chemists*, 2001, **59**, 151-162) Transport of the branched-chain amino acids is important in brewing, specifically because the metabolites of these compounds are converted to higher alcohols, which are important flavour compounds in many alcoholic beverages. This paper considers whether the production of higher alcohols during wort fermentation is responsive to branched-chain amino acid metabolism. Addition of valine, isoleucine and leucine to wort resulted in increased assimilation of each amino acid by yeast and increased production of the corresponding higher alcohol. This suggests that production of a higher alcohol is correlated with the assimilation of the corresponding amino acid. The constitutive expression of the gene (*BAP2*) for the branched-chain amino acid permease in brewing yeast has been studied, focussing on its influences on the production of higher alcohols during wort fermentation. Constitutive expression of the *BAP2* gene resulted in accelerated rates of leucine, valine and isoleucine assimilation. This caused increased production of isoamyl alcohol derived from leucine, while an increase in isobutyl alcohol derived from valine or isoamyl alcohol from isoleucine was not observed. The results contained in this paper support the view that there are distinct but interrelated mechanisms for the production of each higher alcohol.

G.G.S.

***Saccharomyces cerevisiae* Commits to a Programmed Cell Death Process in Response to Acetic Acid.** P. LUDOVICO, M. J. SOUSA, M. T. S. SILVA, C. LEO and M. CORTE-REAL (*Microbiology*, 2001, **147**, 2409-2415) Exposure of *S. cerevisiae* for 3 h to different concentrations of acetic acid (20–200 mM) at a standard pH 3.0 showed complete cell death above 50 mM. Survival was substantially improved over the lower part of the range (50–80 mM) by the addition of 100 µg/mL cycloheximide,

an inhibitor of protein synthesis, indicating that the death of the cells was an active process.

I.C.

**New Aspects of the Glucose Activation of the H<sup>+</sup>-ATPase in the Yeast *Saccharomyces cerevisiae*.** M.A.A. SOUZA, M.J. TROPICA and R.L. BRANDAO (*Microbiology*, 2001, **147**, 2849-2855) Plasma membrane ATPase of *S. cerevisiae* controls an important physiological process: by pumping protons it regulates intracellular pH and provides the driving force for active transport of nutrients. It had previously been shown that glucose-induced activation of plasma membrane H<sup>+</sup>-ATPase occurred by an independent mechanism of cATP signalling, but *RGT 2p* and *SNF 3p* were also shown to be involved: these act as sensors for high and low levels of glucose respectively. Also, sugar phosphorylation was shown to be essential for activation of H<sup>+</sup>-ATPase.

I.C.

**Destruction of Microorganisms Harmful to Beer by High Pressure Treatment.** H.M. ULMER, M.G. GANZLE, and R.F. VOGEL, (*Monatsschrift für Brauwissenschaft*, 2002, **55**, No 1/2, 4-9) Inactivation of lactobacilli by high hydrostatic pressure was studied. It was shown that the pressure-induced destruction of lactobacilli, that are harmful to beer, was possible at pressures from 200 to 600 MPa. Pressure inactivation was considerably accelerated at lower pH values and in the presence of hop bitter substances and alcohol. Dissolved CO<sub>2</sub> only had a slight effect on *L. plantarum*, and sterilisation of a model beer was achieved with liquid CO<sub>2</sub> at a pressure of 12 MPa. Before cell death, caused by high pressure treatment, inactivation of the hop resistant *L. plantarum* was observed. The pressure-induced loss of hop resistance, lead to the loss of ability by *L. plantarum* to survive during beer storage. Sublittoral damage to the cells in the beer was sufficient that no destruction of the organisms was necessary. The lactic acid bacteria were selectively destroyed in a mixture of *S. cerevisiae* and *L. plantarum* using pressure treatment and storage in the presence of hops.

T.B./R.E.W.

#### 4) Processing—Yeast Handling

**The Response of Brewers' Yeast to a Defined Shear Field for Differing Exposure Times.** R.A. STAFFORD, T. STOUPIS and G.G. STEWART (*Proceedings of the European Brewery Convention, Budapest*, 2001, 326-333) Yeast processing between harvest and re-pitching could exert mechanical and hydrodynamic shear stress, and it is likely that the effects of mechanical agitation, pumping, centrifugation and flow through heat exchangers and pipe-work have been underestimated. Ale yeast was collected in a production brewery two and nine generations (fermentations) after first propagation and lager yeast after two and five successive fermentations. All yeast slurries were collected in beer of 5.0–5.3% abv and were adjusted to 50 ± 2% (w/w). To simulate normal yeast agitation, 1.5 litres of slurry was mixed in a baffled 2-litre fermenter with a dual six-bladed turbine stirrer at 500 or 900 rpm. Over 20 min at 500 rpm, viability measured by methylene violet fell by

0.8–1.8% irrespective of yeast type or age. At 900 rpm the loss of viability increased to 3.5% with the ninth generation ale yeast, but the other samples showed only a slightly greater loss of viability than at 500 rpm. Although agitation, especially at 900 rpm, consistently increased the pH of the slurry, again it was the older ale yeast which showed greatest change, by 0.45 pH unit. Also, the higher the shear stress, the greater the liberation of protease, suggesting damage to the cell membrane. It was concluded that, in general, ale yeast was more susceptible to shear stress than lager yeast although this was not confirmed by the approximately equal leakage of protease.

I.C.

**Yeast Membrane Potential and Carbohydrate Utilisation.** S. M. VAN ZANDYCKE, R. SIDDIQUE and K. A. SMART (*Proceedings of the European Brewery Convention, Budapest*, 2001, 334-343) A simple measurement of yeast vitality is acidification power: cells suspended in water and then in glucose solution secrete H<sup>+</sup> ions to equilibrate with the medium. Effectively, the method tests the activity of H<sup>+</sup>-ATPase. Passive H<sup>+</sup> efflux in water is less reproducible than in glucose, but that preliminary stage of 10 min incubation is essential to equilibrate the pH of the cells. Until now the method has used glucose, but maltose is the major carbohydrate of wort; also fructose could be present in significant amount with a fructose or sucrose adjunct. In testing alternative sugars, the fall in pH with fructose and glucose was similar but in maltose the rate of fall was halved. Pre-culture in a maltose medium did not affect the pH results. The difference is explained by the entry of the monosaccharides by facilitated diffusion whereas maltose requires active transport. Yeast from high-gravity (HG) fermentations is unreliable for re-pitching. The structure of the cell membrane is adversely affected by the high alcohol concentrations, which was demonstrated by the effect of 6 h incubation in ethanol before the viability test. Even 4% approximately halved the rate of H<sup>+</sup> efflux, and a further reduction to 25% of the original level occurred by 8–12% ethanol, according to strain. The maximum inhibitory effect was reached by 10–12%, although tests continued without further loss of vitality up to 20% ethanol. Glycogen and trehalose content have also been suggested as tests of yeast vitality. A positive correlation was shown between passive H<sup>+</sup> efflux (i.e., in water) and glycogen content, but no such relationship existed with H<sup>+</sup>-ATPase (i.e., H<sup>+</sup> efflux after addition of glucose) or at any time with trehalose.

I.C.

#### 5) Miscellaneous

**Prospective Study of Moderate Alcohol Consumption and Risk of Hypertension in Young Women.** R. THADHANI, C.A. CAMARGO, M.J. STAMPFER, G.C. CURHAN, W.C. WILLETT and E.B. RIMM. (*Arch. Intern. Med.* 2002, **162**, 569-574) A study funded by the NIH looked at the association between alcohol consumption and subsequent risk of hypertension among 70,891 women, age 25–42 years, who participated in the Nurses' Health Study. During the eight years of follow-up, 4,188 cases (5.9%) of incident hypertension were reported. The researchers studied whether they developed hypertension,

taking into account other factors known to be associated with high blood pressure, such as weight, physical activity, age, smoking and the use of oral contraceptives. The study drew the conclusion that the association between alcohol consumption and risk of chronic hypertension in young women followed a J-shaped curve, with light drinkers demonstrating a modest decrease in risk and more regular heavy drinkers demonstrating an increase in risk. It appears that women who have a few alcoholic drinks a week have an almost 15% lower chance of developing high blood pressure than teetotallers. For beer a drink was defined as a 12-oz bottle. Light beer drinking seemed to be the most beneficial form of alcohol in reducing the risk of high blood pressure, although these preliminary findings need to be confirmed with additional studies.

I.R.

## 6) 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.

**Malt Beverages.** D.E. QUAIN, I. MENNEER, and S.W. MOLZAHN, BRANDBREW, S.A. (LU) (*Patent Number WO0192459, December, 2001*) Beer in a barrel (1) is dispensed via supply line (2) to a dispense tap (3). A light source (5) provided between the barrel (1) and the tap (3) illuminates the beer with light having a wavelength of between 350 and 500 nm in order to cause the beer deliberately to become light-struck. That is to say deliberate irradiation of the beer prior to dispense causes the production of 3-methyl-2-butene-1-thiol (MBT) and this improves the post-dispense flavour stability of the beer since it is no longer liable to change flavour if left in the sun.

**Method of Producing a Composite Fermented Beverage Using Genetically Modified Yeast Strains.** C. GJERMANSEN, J.HANSEN, P.F. JOHANNESSEN, M.B. PEDERSEN, and S.B. SORENSEN, CARLSBERG A/S (COPENHAGEN, DK) (*United States Patent 6,326,184, December 2001*) Method of preparing a composite yeast fermented beverage such as beer including lager, with predetermined content of flavour compounds, comprising combining separate batches of beverage, of which at least one is a base beverage produced with a yeast strain having reduced or lacking production of one or more flavour compounds or flavour stabilizing compounds. In the method are used yeast strains, including *S. cerevisiae* and *S. carlsbergensis*, which have reduced or lacking production of sulphite, dimethylsulphide, thiols, thioesters, hydrogen sulphide, higher alcohols including isoamyl alcohol and/or alcohol esters.

I.R.

**Method for Decontaminating Yeast.** M.C., BARNEY, K.M. CARRICK, A. NAVARRO, and D.S. RYDER, MILLER BREWING COMPANY (MILWAUKEE, WI).

(*United States Patent 6,326,185, December, 2001*) An improved method for reducing colony forming units bacteria in yeast is disclosed. The method involves contacting the yeast with a hop acid in an amount sufficient to give a final concentration of the hop acid of at least about 40 ppm.

I.R.

**Anti-Arteriosclerotic Food.** J.YAMAKOSHI, M. SAITO, A. OBATA, T. IZUMI, and K. TOBE, KIKKOMAN CORPORATION (CHIBA-PREF, JP) (*United States Patent 6,264,997, July, 2001*) A proanthocyanidin and an isoflavone are incorporated into a food. Compounding of grape seed extract as a proanthocyanidin and soy sauce cake extract as an isoflavone gives an anti-arteriosclerotic composition that is effective at a small intake.

I.R.

**Beverage Manufacture and Cold Aseptic Bottling Using Peroxyacid Antimicrobial Composition.** F.L. RICHTER, B.R. CORDS, M.E. BESSE, and K. NOGAMI, ECOLAB INC. (ST. PAUL, MN) (*United States Patent 6,326,032, December, 2001*). A peroxyacid antimicrobial concentrate and use composition is provided comprising a C<sub>1</sub> to C<sub>4</sub> peroxycarboxylic acid or a C<sub>1</sub> to C<sub>4</sub> peroxycarboxylic acid combined with a C<sub>6</sub> to C<sub>18</sub> peroxyacid in beverage processing. The combination of these materials produces a synergistic effect, providing a much more potent biocide than can be obtained by using these components separately. Other components can be added to the composition such as hydrotrope coupling agents, stabilizers, etc. An effective antimicrobial use solution is formed at low concentrations when the concentrate composition is diluted with water to a pH in the range of about 2–8. Sanitizing of substantially fixed, “in-place” processing lines in dairies, breweries, and other food and beverage processing operations is one utility of the composition. Another utility is in processes including aseptic cold filling of beverage containers such as cans, glass bottles or 2-litre PET bottles.

I.R.

**Antimicrobial Activity of Hops Extract against *Clostridium botulinum*, *Clostridium difficile* and *Helicobacter pylori*.** E.A. JOHNSON, and G.J. HAAS, S. S. STEINER, INC. (NEW YORK, NY) (*United States Patent 6,251,461, June, 2001*) The present invention relates to the discovery that hop extract is useful as an antibacterial agent against the dangerous pathogens *Clostridium botulinum*, *Clostridium difficile*, and *Helicobacter pylori* at levels below that at which a flavour from the acids contained therein is objectionable. More specifically, a process and associated product is described herein, comprising applying a solution of hop extract to a food, beverage or other medium so that the final concentration of hop ingredients is about 1 ppm or higher in order to inhibit the growth of *Clostridium botulinum*, *Clostridium difficile*, and/or *Helicobacter pylori*.

I.R.

**Analytical Method and Apparatus.** P.M. VADGAMA, and I.M. CHRISTIE, SENSALYSE HOLDINGS LIMITED (*United States Patent Application 2,002,002,849, Febru-*

ary, 2002) Method and apparatus for detecting and/or determining ethanol in fluid samples using a substantially non-porous barrier of un-plasticised polyvinyl chloride (PVC) interposed between the sample to be analysed and a detecting means responsive to ethanol. The ethanol diffuses through the barrier membrane and then is measured at the detecting means. The PVC membrane can be made by solvent casting, and is usually 10- to 40- $\mu$ m thick. Measurement can be by any known means, but preferably electrochemically (amperometrically). The PVC membrane may be part of a multiple membrane system. The method and sensor are useful for analysing alcoholic liquors or beverages (for example beer, wine and other fermentation products), in their final form or at intermediate stages of their manufacture or storage, and also for the monitoring of a wide range of process, waste and effluent liquids.

I.R.

**Bittering of Beer.** R.J.H. WILSON, and R.J.SMITH, S.S., STEINER, INC. (*United States Patent Application 2,002,0018,840, February, 2002*) Iso- $\alpha$ -acids and reduced iso- $\alpha$ -acids in their free acids states are converted into mobile resins by the addition of concentrated solutions of alkali metal hydroxides. The products may be used in brewing for the bittering of beer and are most effectively used in an apparatus that automatically blends the product with water and injects the resultant, aqueous solution into beer.

I.R.

**Dihydro and Hexahydro Isoalpha Acids Having a High Ratio of Trans to Cis Isomers, Production Thereof, and Products Containing the Same.** K. SHAHLAI, R.H. MENNETT, P.H. TODD, and J.A. GUZINSKI (*United States Patent Application, 2,0020,007,084, January, 2002*) This invention describes heretofore unknown forms of dihydro (DHIA) and hexahydro (HHIA) isoalpha acids having a high ratio of trans to cis isomers and a process for their production. Also, non-precipitating clear 5, 10, and 20% and higher aqueous solutions thereof, since they are soluble at room temperature in soft water. This is due to the high ratio of trans to cis isomers. Unlike prior art essentially all cis isomer products, they remain haze free both at a neutral pH in water and at 1-2% and higher concentrations. This invention has the advantage over the prior art in that DHIA and HHIA can be provided as stable, non-separating liquids, at practical concentrations in the range of 5% to about 40%, which do not require heating to about 50-90°C and above with stirring to effect dissolution of precipitates. The high trans products described herein can be admixed with isoalpha- and tetrahydro-isoalpha acids.

I.R.

**Process for the Production of Beer-like Carbonated Alcoholic Beverage.** K.SHIMAMURA, T. HOZUMI, and T. SASAKI, HOKKAIDO WINE CO., LTD. (*United States Patent Application, 2,0010,043,965, November, 2001*) A beer-like carbonated alcoholic beverage rich in the characteristic flavour of an organic acid such as malic acid or citric acid is obtained by conducting with a Rhizopus or white-Aspergillus mould the saccharification of malt in beer brewing. The formation of the organic acid during the production process makes it possible to allow the fermentation to proceed stably. Furthermore, the beverage obtained according to the present invention has a high alcohol content and has hence been improved in storage stability. When moto is used as a yeast, a beer-like carbonated alcoholic beverage added with sake flavour can be obtained. When a must of fruit wine is employed, a beer-like carbonated alcoholic beverage added with the flavour of a fruit wine can be obtained.

I.R.

**Method for Identifying a Barley Variety and a Barley Having a Brewing Property.** M. KIHARA, T. KANEKO, K. FUKUDA, and K. ITO (*United States Patent Application, 2,0020,019,994, February, 2002*) A method for identifying barley with good brewing properties using the thermostability of the barley  $\beta$ -amylase as an indicator. The thermostability of the barley  $\beta$ -amylase significantly affects the degree of the apparent attenuation limit. A method for determining the enzyme activity of an extract solution from one barley seed, an indirect method by an isoelectric point, and an indirect identifying method by DNA polymorphisms of the region containing the  $\beta$ -amylase structural gene have been developed as a method for determining the type of thermostability for a barley  $\beta$ -amylase. The selection method is not affected by environmental or climatic conditions.

I.R.

**Method and Apparatus for Reducing Foaming during Fermentation.** D.G. BROWN (*United States Patent Application 2,002,0018,840, February, 2002*) The concentration of gas generated in an anaerobic fermenting liquid is controlled, preferably maintained below saturation, by removal of dissolved gas by diffusion during at least part of the fermentation. The removal of gas reduces the amount of foam produced by the fermentation and provides a source of gas for downstream treatment of a fermentation product or export from the fermentation process. The invention has particular application to fermentation processes generating carbon dioxide, especially brewing beer.

I.R.