

# 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

**Xanthohumol in Beer – Possibilities and Limitations of Enrichment.** A. FORSTER, A. GAHR, M. KETTERER, B. BECK, and S. MASSINGER (*Monatsschrift für Brauwissenschaft* 55, No 9/10, 184–194, 2002).

Xanthohumol, a polyphenol of hops, is said to have positive physiological properties. Adequate research, particularly on living cells, has not yet been completed, but it still might be of interest, to establish whether and how xanthohumol may be enriched in beer. Besides conventional pellets, or spent hops after CO<sub>2</sub>-extraction, there are already especially developed xanthohumol extracts available. However, there are limits to the elevation of xanthohumol levels and its isomer isoxanthohumol in commercially filtered beers. Thus, xanthohumol and especially isoxanthohumol levels in, for example bottom fermented commercial beers, differ on a low scale from less than 0.1 ppm or rather less than 1 ppm. At the end of the beer production process only 10–20% of the amount of xanthohumol that has been spiked into wort can be found. If xanthohumol is to be enriched effectively, a dosage with special xanthohumol extracts after fermentation, or yeast filtration, is recommended, whereby a constant turbidity has to be anticipated. Thus, beers rich in xanthohumol/isoxanthohumol are currently only imaginable as specialities. Beyond that, xanthohumol extracts can be added to any kind of turbid and lightly bitter beverages, which on their part can be the basis of beer mix beverages.

TB / REW

### 2) Beer Analysis

**Determinations of Yeast Ageing by Flowcytometric Analysis.** K.-J. HUTTER and F. NITZSCHE (*Monatsschrift für Brauwissenschaft* 55, No 9/10, 196–199, 2002).

In recent years, the bio-monitoring of yeast has been gaining more and more significance. Besides already existing possibilities of characterising the population using cell cycle analysis, the determination of the glycogen level and other intracellular macromolecules in single yeast cells, and determining the bud scar fluorescence helps in evaluating the pitching yeast.

TB / REW

### 3) Brewing – Fermentation

**High Gravity Brewing – an Inducer of Yeast Stress.** P. L. PRATT-MARSHALL, S. E. BREY, S. D. DE COSTA, J. H. BRYCE and G. G. STEWART (*Brewers' Guardian*, 2002, **131/3**, 22–26).

This paper reviews various effects on the yeast of the high sugar or ethanol concentrations associated with high-gravity (HG) brewing. For example, the average volume of the yeast cells increases by up to 30% with an increase in original gravity from 7.5° to 17.5°P, but viability is significantly lower when yeast is grown in HG wort. High osmotic pressure, (caused by either the normal wort sugars of HG wort or the equivalent concentration of non-metabolisable sorbitol), causes wrinkling of the yeast cell wall, and this effect is shown even more strongly in 10% ethanol than in the 20% sugar concentration of 20°P wort. Lager yeasts were more sensitive than ale strains, shown by measuring cell volume and viability throughout a series of experimental fermentations with increasing sugar or ethanol concentration.

Another aspect of yeast osmotic or ethanol stress is the release of proteinase A (PrA) which, by hydrolysis of foam polypeptides, is partly responsible for the poorer head formation and retention associated with HG brewing. HG worts are known to contain approximately 40% less of the hydrophobic polypeptides associated with stable foam. Worse, the excretion of PrA in 20°P fermentations is approximately 5 times the rate at 10°P. In fermentations with deliberate addition of ethanol, the rate and amount of PrA excretion in 10% was approximately twice that in 5% ethanol. Also, the higher pitching rate for HG rather than normal gravity fermentations also increased PrA production. While no remedy is yet available, it is suggested that the effect of osmotic or ethanol stress on plasma membrane ATPase, although known to inhibit nutrient transport, increases PrA excretion.

I.C.

### 4) Microbiology

**Phenotypic Homogeneity: Differential Stress Resistance among Individual Cells of the Yeast *Saccharomyces cerevisiae*.** E. R. SUMNER and S. V. AVERY (*Microbiology*, 2002, **148**, 345–351).

Phenotypic homogeneity, non-genetic variation between individual cells in a genetically pure culture, has important implications in industrial-scale populations of yeast. This paper provides a comprehensive review of cell-to-cell variation in cell ageing, cell cycle progression and mitochondrial activity using stress sensitivity or resistance as a marker.

I.C.

**Effect of Pulsed Electric Fields on Inactivation and Metabolic Activity of *Lactobacillus plantarum* in Model Beer.** H. M. ULMER, V. HEINZ, M. G. GAENZLE, D. KNORR and R. F. VOGEL (*Journal of Applied Microbiology*, 2002, **93**, 326–335).

Pulsed electric field (PEF) treatment is a possible non-thermal alternative to pasteurisation; its lethal effect is believed to be structural damage to the cytoplasmic membrane. Malt extract medium fermented by *Saccharomyces carlsbergensis*, with and without hop extract, was used as the experimental beer in which *L. plantarum* was grown overnight at 30°C. No permanent lethal effect was shown below 19 kV/cm (corresponding to an energy input of 42 kJ/kg) since cell damage at lower values was reversible. Higher-voltage treatment was more effective at lower pH (3.6 rather than 4.0), with isomerised hop extract added to 100 ppm, or nisin at 1 ppm. The results are encouraging for the possible use of PEF in the preservation of beer.

I.C.

**The Hexose Transporters of *Saccharomyces cerevisiae* Play Different Roles during Enological Fermentations.** K. LUYTEN, C. RIOU and B. BLONDIN (*Yeast*, 2002, **19**, 713–726).

The hexose transporter genes *HXT1-7* of an industrial wine yeast strain were investigated individually and in various combinations for effectiveness in fermentation conditions. Fermentation was measured by the rate of release of CO<sub>2</sub>. Inability to ferment hexose sugars when all seven genes were deleted (*HXT1-7Δ*) confirmed that no other hexose transporters were available, although the *HXT1-7Δ* strain was still able to grow on, and ferment, maltose, which is transported by a different mechanism. Of the strains with a single HXT replacement into *HXT1-7Δ*, *HXT3* was the most effective, followed by *HXT1*. *HXT1* or *3* were the only carriers approximating to a normal fermentation, although their effect was weaker than normal at low glucose concentration (0.1%) in the test medium. Conversely, although *HXT2* and *7* (separately) were only weakly effective in 20% glucose they were very effective transporters at 1.0 and 0.1%. The most successful experimental fermentations were achieved by replacing a combination of carriers, e.g. by replacing *HXT1*, *4* and *5*.

I.C.

**Antifungal Activities of Two *Lactobacillus plantarum* Strains against *Fusarium* Moulds *in vitro* and in Malt-ing of Barley.** A. LAITILA, H.-L. ALAKOMI, L. RAASKA, T. MATTILA-SANDHOLM and A. HAIKARA (*Journal of Applied Microbiology*, 2002, **93**, 566–576).

Growth of lactic bacteria on germinating barley is known to inhibit growth of filamentous fungi. This report

concerns the anti-fungal effects of broth culture filtrates of *L. plantarum* on *Fusarium* cultures *in vitro* and of seeding growing cultures into laboratory-scale malting of barley naturally contaminated with fusaria. Cell-free culture filtrates of the test lactic bacteria were added to spore suspensions of *F. avenaceum*, *F. culmorum*, *F. graminearum* or *F. oxysporum* in the cuvettes of an automatic growth analyser. Lactic acid alone could not account for the observed inhibition of fungal growth: the maximum inhibitory effect of 0.25% lactic acid was 20%, against only one of the test strains of *F. avenaceum*, and the effect was negligible against the other mould cultures. However, culture filtrates of either strain of *L. plantarum* showed 33–50% inhibition, depending on the mould strain. Similar effects were observed whether the growth analyser was set to measure turbidity or impedance. For measurement of mould inhibition in experimental malting the whole bacterial culture was added to the steep water. Since fusaria grow rapidly in the first hours of steeping, early addition of the *Lactobacillus* culture was important. Although large variations were noted in the results, which could be explained by the varying level of natural contamination by fusaria, a reduction of 30–50% was noted in *Fusarium* counts on the malt.

I.C.

**Orchestrating the Cell Cycle in Yeast: Sequential Localisation of Key Mitotic Regulators at the Spindle Pore and the Bud Neck.** V. J. CID, J. JIMENEZ, M. MOLINA, M. SANCHEZ, C. NOMBELA and J. W. THORNER (*Microbiology*, 2002, **148**, 2647–2659).

This detailed review article is mainly concerned with the genetic control in *Saccharomyces cerevisiae* of (a) the localisation of the bud at the start of the cell cycle and (b) the biochemical activities at the area of contact of the developing bud with its mother cell.

I.C.

***HXT5* Expression is Determined by Growth Rates in *Saccharomyces cerevisiae*.** R. VERWAAL, J. W. G. PAALMAN, A. HOGENKAMP, A. J. VERKLEIJ, C. T. VERRIPS and J. BOONSTRA (*Yeast*, 2002, **19**, 1029–1038).

Hexose transporter (*hxt*) proteins control facilitated diffusion of glucose across the plasma membrane of *S. cerevisiae*. Although *HXT5* is not essential, since other *hxt* proteins are also available, it is one of the important glucose transporters. However, measurement of *HXT5* activity in continuous culture showed that, contrary to expectation, *HXT5* activity was not influenced by the glucose concentration, but by the growth rate of the yeast cells.

I.C.

**Fourier-Transform Infrared Microspectroscopy, a Novel and Rapid Tool for Identification of Yeasts.** M. WENNING, H. SEILER and S. SHERER (*Applied and Environmental Microbiology*, 2002, **68**, 4717–4721).

Traditional identification of yeasts is achieved by applying physiological and morphological tests, which determine enzyme production profiles and growth characteristics. Various rapid and in some cases automated identification systems for routine analysis of yeasts are commercially available and easy to use. However, identification

results are questionable to a certain degree because these systems were largely developed for clinical application and databases therefore do not include an adequate number of common environmental yeast species. In recent years, many techniques that identify yeasts have been developed. However, their application in the routine analysis of yeasts in the food industry is limited by their high cost and the requirement for highly skilled personnel. A rapid and inexpensive method to identify microorganisms is Fourier-transform infrared (FT-IR) spectroscopy and has been employed in this paper to identify yeasts. Cells were grown to microcolonies of 70 to 250  $\mu\text{m}$  in diameter and transferred from the agar plate by replica stamping to an IR-transparent ZnSe carrier. IR spectra of the replicas on the carriers were recorded using an IR microscope coupled to an IR spectrometer, and identification was performed by comparison to reference spectra. The method was tested by using small model libraries comprising reference spectra of 45 strains from 9 genera and 13 species, recorded with both FT-IR microspectroscopy and FT-IR macrospectroscopy. The results show that identification by FR-IR microspectroscopy is equivalent to that achieved by FR-IR macrospectroscopy but the time-consuming isolation of the organisms prior to identification is not necessary. Therefore, this method provides a rapid tool to analyse mixed populations. Furthermore, identification of 21 *Debaryomyces hansenii* and 9 *Saccharomyces cerevisiae* strains resulted in 92% correct identification at the strain level for *S. cerevisiae* or 91% for *D. hansenii* which demonstrates that the resolution power of FT-IR microspectroscopy may also be used for strain typing at the strain level.

G.G.S.

**Isolation and Characterization of a Freeze-Tolerant Diploid Derivative of an Industrial Baker's Yeast Strain and Its Use in Frozen Doughs.** A. TEUNISSEN, F. DUMORTIER, F.-F. GORWA, J. BAUER, A. TONSHE, A. LOIEZ, P. SMET, P. van DIJCK and J.M. THEVELEIN (*Applied and Environmental Microbiology*, 2002, **68**, 4780–4787).

Frozen doughs are of increasing importance in the bakery sector. These doughs permit the separation of the processes of dough production and baking and also permit large-scale production and distribution of doughs in frozen form independent of the subsequent baking process. The routine production and storage of frozen doughs are still problematic. Although commercial baker's yeast is highly resistant to environmental stress conditions, it rapidly loses stress resistance during dough preparation due to the initiation of fermentation. As a result, the yeast loses gassing power significantly during storage of frozen doughs. Freeze-tolerant mutants of polyploid industrial strains following screening for survival in doughs prepared with UV-mutagenized yeast have been obtained and subjected to 200 freeze-thaw cycles. Two strains in the S47 background with a normal growth rate and the best freeze tolerance under laboratory conditions were selected for production in a 20-liter pilot fermentor. Before frozen storage, the AT25 mutant produced on the 20-liter pilot scale had a 10% higher gassing power capacity than the S47 strain, while the opposite was observed for cells produced

under laboratory conditions. AT25 also retained more freeze tolerance during the initiation of fermentation in liquid cultures and more gassing power during storage of frozen doughs. Other industrially important properties (yield, growth rate, nitrogen assimilation, and phosphorus content) were very similar. AT25 had only half of the DNA content of S47, and its cell size was much smaller. Several diploid segregants of S47 had freeze tolerances similar to that of AT 25 but inferior performance for other properties, while an AT25-derived tetraploid, TAT25, showed only slightly improved freeze tolerance compared to S47. When AT25 was cultured in a 20,000-liter fermentor under industrial conditions, it retained its superior performance and thus appears to be promising for use in dough production. The results also show that a diploid strain can perform at least as well as a tetraploid strain for commercial baker's yeast production and usage.

G.G.S.

**Molecular Analysis of Maltotriose Transport and Utilization by *Saccharomyces cerevisiae*.** R.E. DAY, P.J. ROGERS, I.W. DAWES and V.H. HIGGINS (*Applied and Environmental Microbiology*, 2002, **68**, 5326–5335).

The three principal sugars available to *Saccharomyces cerevisiae* during the fermentation of alcoholic beverages are glucose, maltose and maltotriose. Efficient fermentation requires the rapid and complete utilization of all of these sugars. Maltotriose, which is the second most abundant sugar in wort, is formed from the breakdown of complex sugars during mashing. Efficient fermentation of maltotriose is a desired property of *S. cerevisiae* for brewing. In a standard wort, maltotriose is the second most abundant sugar, and slower uptake leads to residual maltotriose in the finished product. The limiting factor of sugar metabolism is its transport, and there are conflicting reports on whether a specific maltotriose permease exists or whether the mechanisms responsible for maltose uptake also carry out maltotriose transport. In this study, radio-labeled maltotriose was used to show that overexpression of the maltose permease gene, *MAL61*, in an industrial yeast strain resulted in an increase in the rate of transport of maltotriose as well as maltose. A strain derived from W303-1A and lacking any maltose or maltotriose transporter but carrying a functional maltose transport activator (*MAL63*) was developed. By complementing this strain with permeases encoded by *MAL31*, *MAL61*, and *AGT1*, it was possible to measure their specific transport kinetics by using maltotriose and maltose. All three permeases were capable of high-affinity transport of maltotriose and of allowing growth of the strain on the sugar. Maltotriose utilization from the permease encoded by *AGT1* was regulated by the same genetic mechanisms as those involving the maltose transcriptional activator. Competition studies carried out with two industrial strains, one not containing any homologue of *AGT1*, showed that maltose uptake and maltotriose uptake were competitive and that maltose was the preferred substrate. These results indicate that the presence of residual maltotriose in beer is not due to a genetic or physiological inability of yeast cells to utilize the sugar, but rather to the lower affinity for maltotriose uptake in conjunction with deteriorating conditions present at the later stages of fermentation. Molecular mechanisms regu-

lating the uptake of maltotriose are identified. The role of each of the transporter genes in the cells is determined.

G.G.S.

**Membrane-Bound ATPase Contributes to Hop Resistance of *Lactobacillus brevis*.** K. SAKAMOTO, H.W. van VEEN, H. SAITO, H. KOBAYASHI and W.N. KONINGS (*Applied and Environmental Microbiology*, 2002, **68**, 5374–5378).

The primary use of hops in brewing is because of their contribution to the bitter flavour of beer. In addition, the use of hops is preferred because they possess antibacterial activity and prevent bacterial spoilage. Hop compounds are weak acids, which can cross cytoplasmic membranes in undissociated form in response to the transmembrane pH gradient. Due to the higher internal pH, these compounds dissociate internally, thereby dissipating the pH gradient across the membrane. As a result of this protonophoric action of hop compounds, the viability of the exposed bacteria decreases. Some bacteria, however, are able to grow in beer in spite of the presence of hop compounds. The activity of the membrane-bound H<sup>+</sup> ATPase of the beer spoilage bacterium *Lactobacillus brevis* ABBC45 increased upon adaptation to bacteriostatic hop compounds. The ATPase activity was optimal around pH 5.6 and increased up to fourfold when *L. brevis* was exposed to 666 μM hop compounds. The extent of activation depended on the concentration of hop compounds and was maximal at the highest concentration tested. The ATPase activity was strongly inhibited by *N,N'*-dicyclohexylcarbodiimide, a known inhibitor of F<sub>0</sub>F<sub>1</sub> ATPase. Western blots of membrane proteins of *L. brevis* with antisera raised against the α- and β-subunits of F<sub>0</sub>F<sub>1</sub> ATPase from *Enterococcus hirae* showed that there was increased expression of the ATPase after hop adaptation. The expression levels, as well as the ATPase activity, decreased to the initial nonadapted levels when the hop-adapted cells were cultured further without hop compounds. These observations strongly indicate that proton pumping by the membrane-bound ATPase contributes considerably to the resistance of *L. brevis* to hop compounds.

G.G.S.

**Yeast Diversity and Persistence in Botrytis-Affected Wine Fermentations.** D.A. MILS, E.A. JOHANNSEN and L. COCOLIN (*Applied and Environmental Microbiology*, 2002, **68**, 4884–4893).

Numerous studies have examined the succession of yeasts and bacteria that occurs during the fermentation of non-sterile musts. In general, yeasts predominate during the alcoholic fermentation, where the low pH and nutritional content of the juice select for yeast growth. Indigenous non-*Saccharomyces* yeasts and bacteria are potential causes of stuck and sluggish wine fermentations. Winemakers often increase this possibility by seeking to reduce the overall amount of the sulphur dioxide used in winemaking, which is employed, in part, to eliminate indigenous microbial populations. In addition, there has been renewed interest in defining the microbial dynamics of spontaneous (uninoculated) fermentation in order to better understand and control fermentation behaviour and its subsequent impact on wine flavour. Culture-dependent and -independent methods were used to examine the yeast diversity present

in botrytis-affected (“botrytized”) wine fermentations carried out at high (~30°C) and ambient (~20°C) temperatures. Fermentations at both temperatures possessed similar populations of *Saccharomyces*, *Hanseniaspora*, *Pichia*, *Metschnikowia*, *Kluyveromyces*, and *Candida* species. However, higher populations of non-*Saccharomyces* yeasts persisted in ambient-temperature fermentations, with *Candida* and, to a lesser extent, *Kluyveromyces* species remaining long after the fermentation was dominated by *Saccharomyces*. In general, denaturing gradient gel electrophoresis profiles of yeast ribosomal DNA or rRNA amplified from the fermentation samples correlated well with the plating data. The direct molecular methods also revealed a *Hanseniaspora osmophila* population not identified in the plating analysis. rRNA analysis also indicated a large population (>10<sup>6</sup> cells per ml) of a nonculturable *Candida* strain in the high-temperature fermentation. Monoculture analysis of the *Candida* isolate indicated an extreme fructophilic phenotype and correlated with an increased glucose/fructose ratio in fermentations containing higher populations of *Candida*. Analysis of wine fermentation microbial ecology by using both culture-dependent and -independent methods reveals the complexity of yeast interactions enriched during spontaneous fermentations.

G.G.S.

**Intracellular Maltose is Sufficient to Induce *MAL* Gene Expression in *Saccharomyces cerevisiae*.** X. WANG, M. BALI, I. MEDINTZ and A. MICHELS (*Eukaryotic Cell*, 2002, 1, No 5, 696–703).

The presence of maltose induces *MAL* gene expression in *Saccharomyces* cells, but little is known about how maltose is sensed. Strains with all maltose permease genes deleted are unable to induce *MAL* gene expression. In this study, the role of maltose permease in maltose sensing was examined by substituting a heterologous transporter from the dicot plant *Plantago major* that exhibits no significant sequence homology to maltose permease. When expressed in *Saccharomyces cerevisiae*, PmSUC2 is capable of transporting maltose, albeit at a reduced rate. The introduction of PmSUC2 was shown to restore maltose inducible *MAL* gene expression to a maltose permease-null mutant and that this induction requires the *MAL* activator. These data indicate that intracellular maltose is sufficient to induce *MAL* gene expression independently of the mechanism of maltose transport. By using strains expressing defective *mal61* mutant alleles, it was demonstrated that there was a correlation between the rate of maltose transport and the level of induction, which is particularly evident in medium containing very limiting concentrations of maltose. The results indicate that a rather low concentration of intracellular maltose is needed to trigger *MAL* gene expression.

Constitutive overexpression of either *MAL61* maltose permease or *PmSUC2* was shown to suppress the non-inducible phenotype of a defective *mal13* *MAL*-activator allele, suggesting that this suppression is solely a function of maltose transport activity and is not specific to the sequence of the permease. The studies indicate that maltose permease does not function as the maltose sensor in *S. cerevisiae*.

IR / REW

**Eng1p, an Endo-1,3-β-Glucanase Localized at the Daughter Side of the Septum, is involved in Cell Separation in *Saccharomyces cerevisiae*.** V. BALADRÓN, S. UFANO, E. DUEÑAS, A.B. MARTÍN-CUADRADO, F. del RAY and C.R. VÁZQUEZ de ALDANA (*Eukaryotic Cell*, 2002, 1, No 5, 774–786).

*ENG1* (YNR067c), a gene encoding a new endo-1,3-β-glucanase, was cloned by screening a genomic library with a DNA probe obtained by PCR with synthetic oligonucleotides designed according to conserved regions found between yeast exo-1,3-β-glucanases (Exg1 p, Exg2 p, and Ss1 p). Eng1 p shows strong sequence similarity to the product of the *Saccharomyces cerevisiae* ACF2 gene, involved in actin assembly “in vitro”, and to proteins present in other yeast and fungal species.

It is related to plant glucan-binding elicitor proteins, which trigger the onset of a defence response upon fungal infection. Eng1p and Acf2p/Eng2p are glucan-hydrolysing proteins that specifically act on 1,3-β linkages, with an endolytic mode of action. Eng1 p is an extracellular, heavily glycosylated protein, while Acf2p/Eng2p is an intracellular protein with no carbohydrate linked by N-glycosidic bonds. *ENG1* transcription fluctuates periodically during the cell cycle; maximal accumulation occurs during the M/G<sub>1</sub> transition and is dependent on the transcription factor Ace2p. Interestingly, *eng1* deletion mutants show defects in cell separation, and Eng1 p localises asymmetrically to the daughter side of the septum, suggesting that this protein is involved, together with chitinase, in the dissolution of the mother-daughter septum.

IR / REW

## 5) Beer – Processing

**Fundamentals of Electro Spin Resonance Spectroscopy (ESR), and Research Concerning the Correlation Between Oxidative Beer Stability and Sulphite Content.** T. KUNZ, A. STEPHEN, F.J. METHNER, R. KAPPL and J. HÜTTERMAN (*Monatsschrift für Brauwissenschaft* 55, No 7/8, 140–153, 2002).

It is well established that sulphite is the main component for the antioxidant potential in beer. This endogenous antioxidant buffer capacity for reactive radicals, which is mainly produced by activated oxygen, can quantitatively be determined by the lag time of beer during a forcing test using electro spin resonance spectroscopy (ESR). Detailed lag time measurements of various beer samples with a different sulphite content verify former results, but also show that there is a significant non-linear dependency, whose cause has not been clarified as yet. In connection with the distinct dependency of the lag time on the sul-

phite content of beer, the formation of sulphite complexes in conjunction with staling carbonyls are discussed. The fundamentals of ESR spectroscopy are presented, which are particularly important for the interpretation of the solution spectra of the spin trap radical adducts being formed during lag time measurements.

TB / REW

**Experiences in Measuring Free Radicals using Electro Spin Resonance Spectrometry in the Brewery.** O. FRANZ and W. BACK (*Monatsschrift für Brauwissenschaft* 55, No 7/8, 156–162, 2002).

It is possible to detect free radicals in both wort and beer by means of electro spin resonance spectroscopy (ESR). These radicals are involved in oxidative reactions and thus in staling processes in beer. The lag time, which is determined by ESR, demonstrates the endogenous antioxidant activity of beer. Lag time shows a distinct correlation to the expected flavour stability. Despite the relatively simple application of ESR, there are several factors to consider in order to obtain reproducible analysis results, which are comparable between different brewing laboratories. The extract content, pH and ethanol content of the sample play an important role. When measuring in wort, it is vital to establish extract content and pH. As the number of laboratories using ESR for operations control increases, the experiences of the Chair for Brewery Technology I are shown. In the long run it makes sense to agree on one procedure for preparing samples, and for evaluation of the measurements, to be able to compare the results.

TB / REW

## 6) Patents issued and patent applications

**Method for Identifying a Barley Variety and a Barley having a Brewing Property.** M. KIHARA, T. KANEKO, K. FUKUDA AND K. ITO, SAPPORO BREWERIES LTD. (*United States Patent* 6,492,576, December, 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.

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