Poster debate – Yeast

During the course of the EBC held at Cannes during May and June 1999 a workshop was held to discuss a number of the posters related to yeasts. The workshop was jointly run by David *Ryder* from Miller Brewing and Poul *Sigsgaard* from the Scandinavian School of Brewing. In his introduction David reminded his audience of the importance of yeasts for beer production; happy yeasts will produce beers which delight the brewers' customers whereas unhappy yeasts will lead to problems with both flavour consistency and flavour stability. Five posters had been selected from those displayed at the Congress and their authors were asked to make a short presentation setting out the main findings of their work. In particular, they were asked to answer three questions: What is the primary discovery of your contribution? How important is this in terms of yeast? How far is the work from implementation and use? Encouraged by the chairmen (in some cases forcibly) the small but select band of participants then debated the implications of the work.

Construction of *S. carlsbergensis* brewer's yeast

The first discussion centred around Poster 24: "Construction of *S. carlsbergensis* brewer's yeast" without production of sulphite by Pia Francke *Johannesen*, Carlsberg Research Laboratory. The primary discovery was concerned with producing yeasts which make no sulphite. Two diploid spore clones from brewers yeast had been separately manipulated in order to inactivate the *MET14* genes (one of the genes encoding enzymes on the sulphate to sulphite pathway). The diploids had then been crossed to generate a tetraploid brewers yeast which had all 4 copies of the *MET14* gene inactivated. This yeast had been used in 50 hl pilot fermentations.

The *met14* strain produced virtually no sulphur dioxide whilst the control produced about 10 mg/litre. The *met14* strain also produced about 50% of the level of acetaldehyde. There were however no differences between the levels of trans-2-nonenal produced in the two beers on forced ageing. Thus sulphur dioxide levels affect acetaldehyde but not trans-2-nonenal production. The low sulphite beer after forced ageing was heavily oxidised but the control beer was not.

In terms of yeast, the work had clearly demonstrated that brewing lager yeast contains two copies of each of two homeologous *MET14* genes.

The technology could be implemented immediately except that the low sulphite producing strain has been genetically-modified and so might not be suitable for brewing.

Several debaters questioned the point of doing the work since clearly sulphur dioxide is needed for the prevention of ageing and in some countries the addition of sulphur dioxide is not permitted. Here a yeast that makes sulphur dioxide is required. The author pointed out that the main point of the work had been to see whether sulphur dioxide produced during fermentation is any different from added sulphite. On the other hand since sulphur dioxide is mainly added to mop up oxygen and to get rid of aldehydes (especially acetaldehyde) another debater questioned whether, with good modern brewing practice, sulphur dioxide was needed at all. It was his opinion that yeast which produces large quantities of sulphur dioxide tends to produce unstable beer. To a question on hydrogen sulphide production, the author reported that, as expected, there was also a reduction in the level of this compound on using this yeast. She also confirmed that there were no differences in the uptake of free amino nitrogen or the levels of glycerol when compared to the control. Since the yeast is not producing sulphur dioxide, one member of the audience wondered what else it is producing and how the redox balance is achieved. The presenter had no data concerning this.

Improvement of maltose and maltotriose brewery fermentation efficiency using immobilised cell technology

The second presentation was about Poster 25: "Improvement of maltose and maltotriose brewery fermentation efficiency using immobilised cell technology" by Ronnie *Willaert*, Hogeschool Gent. The primary discovery was that the uptake of maltose by immobilised yeasts is greater than uptake by yeasts in suspension. Using a specially designed membrane reactor containing alginate-immobilised yeasts the presenter had measured the uptake of glucose and maltose and developed a mathematical model to describe the system. Yeast cells located in the depth of the alginate are not repressed by glucose (which has been removed by yeast cells in the outer layer of the gel) and consequently take up maltose at about three times the rate of free floating cells. Thus using gel immobilisation, sugar uptake can be separated in space. Similar effects should occur with maltotriose and amino acid uptake.

In terms of yeast this represents another way of eliminating the glucose repression effect on maltose metabolism. This procedure has the advantage that it does not require genetically altering the yeast.

As for implementation, the model should be useful in optimising immobilised yeast systems although considerable work is still needed to look at diffusion limitations etc.

During the discussion several participants wondered whether the simplest solution may not be to eliminate glucose from brewers worts. An alternative was to ferment glucose only but it was generally considered that this would produce poor quality beer. Regarding the model, one debater felt that it was dangerous to use a model based on a steady state system since the uptake rates may be very different in a real situation. The discussion also encompassed the much wider subject of immobilisation for primary fermentation but it was generally felt that two stage fermentations corresponding to primary and secondary fermentation would be needed, especially if a fast primary fermentation generated beer with large amounts of diacetyl.

The oxidative stress response of ale and lager yeast strains

Next we heard a presentation on Poster 27: "The oxidative stress response of ale and lager yeast strains" – Veronique *Martin*, Oxford Brookes University. Oxidative stress was assessed by measuring resistance of yeast cells to hydrogen peroxide. The main findings were that stationary phase cells from YPD medium were more resistant than exponential phase cells and that growth in wort generally increased the resistance of cells. The rate of death depended upon hydrogen peroxide concentration. Lager yeasts were more resistant than ale yeasts. Catalase levels appeared to be related to the resistance to stress whilst glutathione content was inversely related to catalase activity.

Having established a baseline the work will be expanded to see whether oxidative stress affects fermentation performance. As yet the work is only a preliminary investigation and is far from implementation.

Several members of the audience questioned the likelihood of any negative effects of oxygen on yeast since heavily aerated yeasts from both propagation vessels and continuous fermentation systems appear to behave normally as far as beer production is concerned. Others however felt that in some circumstances over-oxygenation can trigger strange responses and it may be that on recycling such yeast brewers may be storing up problems for themselves. It may also impact on the flavour and flavour stability of the products. Another debater commented on the similarity between these results and those seen during acid washing, particularly when ammonium persulphate, an oxidising agent, is used. Specifically, the difference in sensitivity between exponential phase and stationary phase cells is also seen when yeast cells are acid washed. There followed a general discussion on the methods used for the measurement of viability and in one debaters opinion, other methods of determining viability should be included in the study. One member of the audience commented that it would also be interesting to know the effect of oxidative stress on yeast vitality.

A novel system for propagation of brewing yeast

The fourth presentation was concerned with Poster 30: "A novel system for propagation of brewing yeast" by Chris *Boulton*, Bass Brewers Ltd. The primary novelty described in this poster was the use of a two vessel propagation system in which oxygen is metered using a thermal mass flow meter coupled with a continuous variable speed agitator. Foam is controlled by applying nitrogen top pressure. The yeast cells are not under stress because the oxygen concentration is always below 0.5 ppm.

The system achieves growth from 5 million cells to 200 million cells per ml in about 30 hours, enough to pitch a 1500 hl fermenter. The complete propagation cycle takes as little as 5 days. The yeast is of consistent physiology and performs well. However the fermentation rates are always significantly slower for the first and second generation fermentations. This is overcome by raising fermentation top temperature by 1 °C. Commercially the system works well and has already been in use for 2 years.

One advantage of this system is that, since oxygenation is well controlled and never exceeds 0.5 mg/litre, there is little chance of the sort of yeast stress described in the preceding presentation. The yeasts ferment well with about 98% viability. There was some concern about the need for nitrogen top pressure for foam control since some yeasts are very pressure sensitive; however this was clearly not the case with the yeasts for which the system was designed. One debater asked if there was an increase of sulphur compounds with the use of top pressure but the author replied that the yeast produced perfectly normal beer. Comments were also made concerning the fact that the use of oxygen rather than air can sometimes produce yeasts which ferment well but which are slow at removing diacetyl at the end of fermentation. Once again the importance of strain to strain variation was emphasised.

The question of the slower initial fermentations was discussed at some length. Explanations included oxidative stress, low pitch rates due to heavily oxygenated cells being smaller than usual and the changed conditions (lower temperature, large vessel, lower oxygen content) in the fermenter. It was generally agreed that lower attenuation rates are nearly always observed in the first fermentation after propagation.

A novel yeast factor – impact on brewing performance

The final discussion was concerned with Poster 31: "A novel yeast factor – impact on brewing performance" by Alain *Debourg*, Institut Meurice. The author explained that he was about to describe a case of cannibalism! A novel Yeast Peptide Factor (YPC) had been extracted from dried yeast using methanol and water followed by purification by gel filtration and ion exchange chromatography. YPC appears to have a molecular weight of about 1500 and contains a number of amino acids. YPC has a positive effect on yeast metabolism, in particular it increases its fermentative capacity, improves its tolerance to ethanol and osmotic stresses and improves its rate of fermentation of high gravity worts. At the same time however it appears to stimulate formation of acetate esters and propanol whilst decreasing formation of other higher alcohols.

Clearly this is of major importance as far as yeast physiology is concerned since YPC improves so many aspects of yeast behaviour. However implementation is not imminent since further purification and characterisation is required coupled with an investigation into the mechanism of action. In particular the nature of the effects needs to be investigated, especially the effects on production of flavour compounds.

Discussions focused on the possible nature of the material. Although methanol had been used in the extraction procedure there appeared to be no lipid in the active YPC fraction. However most of the effects of YPC could be membrane-related and so interactions with lipids should not be ruled out at the moment. At present there is no information on the stage during the yeast life cycle when this material is produced but it can certainly be extracted from both freshly propagated yeast and yeast derived from fermentations. As yet, no attempt has been made to extract the material from active dried yeast or from bakers' yeast.

Although attention had been concentrated on the physiological effects of YPC, one member of the audience pointed out that the major potential commercial benefit could be in the shortening the yeast recycle times in high gravity fermentations. He did however point out that rather large amounts of material are needed for an effect and moreover that the results so far had been achieved with only one yeast and one wort. Further investigation would be needed before the universal use of YPC could be contemplated.

The two chairmen are to be congratulated for organising an interesting and occasionally controversial workshop which was sufficiently stimulating to keep a group of brewing scientists in a lecture theatre on a warm afternoon in Cannes when they could have been sunbathing and drinking a cold beer.

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