High Gravity Wort Clarity and Its Effect on Brewing Yeast Performance

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ABSTRACT

Experiments were designed to assess the impact of wort clarity on the fermentative performance of a production lager yeast Saccharomyces cerevisiae var. uvarum AJL 2036. Cloudy wort as produced within a commercial brewery offered no apparent nutritive value to this yeast strain and its particulate content did not serve to alleviate CO₂ inhibition. Cloudy wort led to poorer yeast growth, slower attenuation and more residual free amino nitrogen in the final beer. All of these factors lead to a poorer quality fresh beer and would decrease beer flavor stability. Some of the yeast inhibition could be removed through the adequate supply of oxygen and the alleviation of CO₂ toxicity. However, full yeast performance could not be restored. It was postulated that cloudy wort particles adhering to yeast cells caused most of the effects. The formation or removal of flavor active substances showed no correlation to wort clarity but reflected overall fermentation rates. The best overall fermentative and yeast performance was observed in clear wort augmented with oxygen and a protein-based yeast stimulant that functioned to lessen CO₂ toxicity. This stimulant also promoted more rapid and complete yeast flocculation in all the treatments assessed. The results of commercial trials comparing the production performance of cloudy versus clear worts are also presented. Again, clear wort resulted in quality and economic improvements.

KEYWORDS: wort clarity, yeast performance, high gravity brewing, nutrition, carbon dioxide toxicity

SINTÉSIS

Se diseñaron experimentos para determinar el impacto de claridad de mosto en el desarrollo de la fermentación de una levadura lager Saccharomyces Cerevisiae Var. Uvarum AJL2036. El mosto turbio como se produce en una cervecería comercial no ofrece un valor nutritivo aparente a esta cepa de levadura y su contenido de partículas no sirvió para aliviar la inhibición de CO₂. En el mosto turbio hubo menos crecimiento de la levadura, atenuación más lenta, y más residuo de amino nitrogeno libre en la cerveza terminada. Todos estos factores llevaron a tener peor calidad de cerveza fresca y puede disminuir la estabilidad del sabor de la cerveza. Algo de la inhibición de la levadura se puede quitar por medio de un suministro adecuado de aire y la disminución de toxicidad del CO₂. Sin embargo, no se pudo rescatar se postulo que partículas de un mosto turbio que se adhieren a las células de levadura son las causantes de la mayoría de efectos. La formacion o retiro de sustancias activas de sabor no demostraron correlación alguna contra claridad del mosto pero reflejaron efecto en los grados de fermentación. El mejor resultado en general en lo referente a fermentación y desempeño de la levadura se observo con mosto claro dosificado con oxígeno y un estimulante de levadura a base de proteína que funcionan para reducir toxicidad del CO₂. Este estimulante también promovió una floculación más completa y rápida de la levadura en todos los ensayos llevados analizados. Los resultados en pruebas a nivel comercial comparando el rendimiento en mosto claro vs. turbio también son presentados. Nuevamente, un mosto claro resulta en mejores cualitativas y económicas.

INTRODUCTION

Brewers have debated for decades the relative merits of producing cloudy versus clear worts. The “cloudy” material consists of lipids, protein-tannin complexes, low molecular weight proteins and insufficiently degraded starch (22,38). Proponents of cloudy wort usually refer to its yeast nutritional benefits (13,20,32,33,35). Cloudy wort does contain more lipid material and, as such, if wort oxygenation is insufficient, the yeast could take up the exogenous lipid from the wort. Yeast cells do this as brewers’ yeast will always contain polyunsaturated lipid once it has been grown in Wort (2,7,8). These yeasts cannot synthesize these lipids so they had to have originated in the wort. However cloudy wort will not contain any ergosterol (11) which is more important to yeast fermentative performance than unsaturated fatty acids (11). Therefore, under-oxygenation cannot be corrected by supplying cloudy wort as an unsaturated fatty acid source (3,4). Additionally, we now know that oxygen fulfills other roles in the yeast cell and cannot be substituted or limited if fermentation performance is to be optimized (21,26). Clear wort will also contain polyunsaturated fatty acids albeit to a lesser degree (7,23,35). Smillie et al (35) found that there was no significant difference in total fatty acids in cropped yeast grown in filtered wort and yeast grown in normal production wort. Free lipids remained in finished beer suggesting that in well oxygenated wort, there is no evidence for a shortage of fatty acids.

Another reported advantage of cloudy wort is the particulate matter contributing CO₂ nucleation sites thus helping to alleviate CO₂ toxicity effects (34). As has been proven (6), this is the basis of a protein-based yeast stimulant our breweries routinely employ. The yeast stimulant was evaluated to derive most of its effects from the toasted, defatted soya flour fraction (6). Since brewers’ yeast cannot use protein as a nitrogen source (11), the effect was not nutritional. The significant response of our com-
commercial lager yeast strain to the addition of this stimulant suggested that CO₂ toxicity was a limiting condition in our fermentations. However, we have found that adding the stimulant above its recommended dosage rate did not result in further effects and, as such, cloudy wort particulate matter might not improve the situation if the yeast stimulant was present. However, the contribution of cloudy wort to CO₂ nucleation cannot be entirely neglected for every brewery situation. Therefore this aspect was researched in this study.

Cloudy worts have been reported to contain more yeast nutrients (non-lipid) than do clear worts (33). This would result in better fermentation performance than in clarified wort. It is impossible to say what these compounds would be either qualitatively or quantitatively as they would vary in nature and amounts according to raw materials and the wort production method (5,10,22,25,35). The effect of the nutrients would also be very yeast strain-specific. Lentini et al (18) suggested that the bioavailability of zinc in cloudy worts could be problematic as it bound very strongly to trub and was not available to yeast. However, they found cloudy wort more stimulatory to fermentation and this was not associated with higher (or lower) cellular zinc concentrations. The predominant mechanism was attributed to the provision of CO₂ nucleation sites. This agrees with research conducted in our laboratories (6) which attributed the effects of particulates to nucleation site provision and negated the contribution of wort zinc contributions for our yeast strain. Residual metal concentrations in finished beer should be minimized due to the catalytic role of metals in promoting chemical and organoleptic changes in beer character (25).

An advantage of cloudy worts that cannot be ignored is that they can be produced more quickly in a brewery. Attention to raking heights and run-off speeds can be lessened with an overall decrease in the cycle time. However, this is not advantageous to beer quality. It must also be stressed that the whirlpool cannot be used to clarify such worts and it is well proven that if cloudy wort enters the whirlpool - cloudy wort will also exit the whirlpool (19,25,38). Poorer wort separation in the whirlpool will also lead to higher wort losses (19,38). Hot break is greatly reduced when boiling turbid wort (25,38).

There are several reported disadvantages in running cloudy wort. It is generally accepted that such wort results in a less flavor stable beer (20,25,35). The lipids that are supposed to act as yeast nutrients undergo reactions that lead to the formation of staling compounds. As a result, product shelf life deteriorates (20,25,38). This point is not entirely accepted, as several researchers have found no relationship between wort lipid content and flavor stability (16,34,35). Staling compounds exist in μg/L concentrations whereas fatty acids can be found in mg/L or even percentage levels. Cause and effect relationships are not easily established due to these sensitivity differences. Most evidence suggests that wort fatty acids do play a role in beer flavor stability. Colloidal stability could also be problematic in beers made from cloudy worts (17,25). Residual lipid material will also lead to foam-negative effects (10,20).

Cloudy wort also causes hygiene and sanitation problems at all stages upstream. Cold protein breaks are larger with concomitant tank cleaning problems. The yeast crop is also much dirtier (20). The problem is not one of underpitching due to trub mass contributions but rather to yeast behavior itself. During fermentation, clean yeast acting as a charged particle will attract oppositely charged wort particles to its surface. Here these particles aggregate and less cell surface area is then available to ferment and excrete by-products. The active surface area for fermentation becomes substantially reduced throughout the fermentor volume. As daughter cells bud from the surface, they too are covered with wort particles.

Zanigrando (38) found that cloudy wort also gave rise to poorer bitter substance utilization figures in ferment (additional 10-20% loss). It is possible that bittering substances associate with particulate matter in the fermenting wort. This will also occur in the kettle leading to additional bitterness loss (25). The economic consequence of this alone is a strong motivator to running clearer worts but substantial savings can also be realized on filter aids and filtration efficiency.

Experiments were designed and conducted to address the above controversy of clear versus cloudy wort. These as well as preliminary commercial trials indicated that wort should be produced as clear as possible in order to ensure that yeast performance is optimized.

**MATERIALS AND METHODS**

**Laboratory Experiments**

**Yeast**

A commercial *Saccharomyces cerevisiae* var. *uvarum* yeast strain was employed. The strain designation was Alfred Jorgensen Laboratory (AJL 2036 (Copenhagen, Denmark). The yeast was collected from the brewery in a presterilized flask and kept cold (4°C) until use (3 h later). The yeast employed for the laboratory-based experiments was recovered from two previous commercial fermentations and had been transferred to the yeast collection vessel and mixed at the time of pick-up. Its performance in previous fermentations was assessed to be normal for this yeast.

**Media**

**Simulated Wort Treatments**

The simulated wort was based on the formulation of Yoshioka and Hashimoto (37) and was prepared and gravity-adjusted as previously described (26). High maltose syrup (African Products Ltd., Johannesburg, S.A.) was used for gravity adjustment from 12° Plato (g sugar measured as sucrose per 100 g wort at 20°C) to 16° Plato (°P).

The treatments assessed were as follows:

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>LIPID</th>
<th>PROTEIN STIMULANT</th>
<th>OXYGEN</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
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<td>+</td>
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<tr>
<td>7</td>
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</tr>
<tr>
<td>8</td>
<td>+</td>
<td>+</td>
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</table>

All 8 treatments were conducted in duplicate. Simulated wort contains no natural source of lipid material and, therefore, is a suitable medium to which to add lipid (oleic acid, ergosterol) and evaluate its nutritive effect. Ergosterol was added at 40 mg/L and oleic acid was supplemented at 0.4% w/v. The standard solutions were made as previously described (11). The pro-
tein stimulant (Fermavite, Syndachem, Johannesburg, South Africa) was added at an optimized concentration of 40 mg/L where required (6). Oxygen (14 mg dissolved oxygen per L wort) was added to the relevant treatments as previously described (30).

**High Gravity Brewery Wort Treatments**

The 16°P wort was 65% malt : 35% adjunct on an extract (w/w) basis. The adjunct was high maltose syrup (African Products Ltd.). All wort was brewed in a 32 L microbrewery using standard procedures. Cloudy wort was produced by lautering quickly (100 min) and recovering the hot wort (85°C) from the whirlpool. Clear wort was lautered for the normal 180 min and recovered cold (10°C) after the paraflow (heat exchanger). It was further filtered (gravity filtration) through Whatman #1 filter paper to produce a brilliantly clear wort. EBC haze values (1) for the different wort types were: cloudy wort = 89.4 EBC units; clear wort (ex: paraflow) = 51.5 EBC haze units; clear (filtered) wort = 1.2 EBC haze units. Only clear (filtered) wort was used in the subsequent treatments.

The treatments assessed were as follows:

<table>
<thead>
<tr>
<th>CLEAR WORT</th>
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<tbody>
<tr>
<td>TREATMENT</td>
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<tr>
<td>-----------</td>
</tr>
<tr>
<td>1</td>
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<tr>
<td>2</td>
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<tr>
<td>3</td>
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<td>4</td>
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<table>
<thead>
<tr>
<th>CLOUDY WORT</th>
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<tbody>
<tr>
<td>TREATMENT</td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td>5</td>
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<tr>
<td>6</td>
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<td>7</td>
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<td>8</td>
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</table>

All 8 treatments were conducted in duplicate. The protein stimulant dosage rate was the same as for the simulated medium as was the oxygenation protocol and level.

**Fermentations**

All the fermentations were conducted in 2L European Brewery Convention (EBC) tubes and the temperature was held at 11°C until completion (two constant gravity readings over 24 h). The inoculation rate was 14 g slurry weight cells per L wort corrected for the viability percentage as measured by methylene blue (15). This procedure resulted in an initial cell count of 23-25 x 10⁶ viable yeast cells per mL of medium. All treatments were inoculated with the same batch of yeast.

**Analytical Assays**

During fermentation, wort samples were removed and processed for analysis. Yeast counts were measured using a Coulter Counter (Model D Industrial, Coulter Electronics, Luton, U.K.). Wort gravity (°P) was determined using the Anton-Paar DMA 55 density meter (Graz, Austria) and converting to °P with EBC charts. Acetaldehyde was measured according to the method of Delcour et al (12). Sulfur dioxide concentrations were determined using the pararosaniline method (1). Diacetyl and 2,3-pentanedione were measured as previously described (6). Alcohol determinations were conducted using the 5600 SCABA automatic beer analyzer (Tecator, Sweden). Free amino nitrogen (FAN) was measured according to the EBC ninhydrin method (14). Dimethylsulfide (DMS) was measured in brewers’ wort samples by the recommended Institute of Brewing procedure (15) except that ethylmethyl sulfide (Fluka Chemicals, Sweden) was used as the internal standard. DMS was not measured in the simulated wort since no precursor would have been present in the absence of malt. Protease was measured using the method of Mochaba et al (24).

**Commercial Trials**

**Brewing Procedure**

The commercial trials were conducted at a 330 hL brewlength plant (United Breweries, Garankuwa, South Africa). Each brew (330 hL) was brewed to a final gravity of 16°P. The malt : adjunct ratio was 65% : 35% on an extract basis with 20% maize and 15% high maltose syrup composing the adjunct. The kettle finings (Biocon, Quest International, Kent, U.K.) were added (4 g/L) 80 min. after initiation of the kettle boil (total boil time = 90 min.). After the kettle boil, the wort was moved hot to the whirlpool where trub was removed via a centrifugal type separation. Yeast stimulant was added at a dosage rate of 4 g/L to all brews. The wort was then cooled (11°C) via a heat exchanger and transferred directly to the fermenter. No cold wort separator was employed. During transfer to the fermenter, the wort was oxygenated (18 mg dissolved oxygen /L wort) and inoculated (1.4 kg yeast slurry per hL wort, 20-25 x 10⁶ cells / mL wort). The entire yeast inoculum was added to the first 330 hL brew. Two brews were pooled per fermenter (660 hL vessel) and both were oxygenated to the same level. Fermentations were temperature controlled to a maximum of 14°C. After the beer gravity had reached the required degree of fermentation, the yeast was harvested from the bottom of the fermenter.

After the beer diacetyl concentration had reached 50 μg/L, the beer was moved to cold storage (1°C) until deemed ready for dilution and filtration. The beer was diluted 40% v/v with cold deaerated carbonated water after filtration. For filtration, a sheet filter (86.3 m², 250 hL/h) was employed and was precoated with diatomaceous earth (Kieselguhr filteraid made up in cold deaerated carbonated water). The beer was dosed with bodyfeed (295 kg coarse and 45.4 kg standard Kieselguhr made up in 30 hL of dilution water dosed in at 1% of filtered volume). During each filter run (trial and control), the mass of Kieselguhr used over the volume filtered was measured and a Kieselguhr usage rate (g/hL) was calculated. After filtration and dilution, the beer was carbonated and moved to the bottling tank. Finished beer was packaged in returnable quart bottles and tunnel-pasteurized for biological stability (15 pasteurization units given, equivalent total heat treatment 15 min. at 60°C).

**In Process and Finished Beer Analyses**

Diacetyl, free amino nitrogen (FAN), gravity and yeast counts were conducted as above. Alcohol was calculated from the density readings and refractive indices. Foam half life and
adhesion were measured using a modified Blom (9) method (Alfred Jorgensen Laboratories, Copenhagen, Denmark). Beer was artificially foamed under standard conditions for the method. The rate of collapse was measured and calculated as a half-life assuming that the foam represented a single reacting species and that all foams behave identically. In this manner, straight line second order reaction relationships between the volume of collapsed foam and the foam half-life were developed.

RESULTS AND DISCUSSION

Simulated Wort Experiment

Previous research employing this medium (26) demonstrated that yeast growth does not achieve the same level as in normal brewers' wort. Some undefined component(s) in wort stimulate yeast growth and was (were) missing from simulated wort. We expected a lower than average (2-3 fold increase) growth level in this medium for all the treatments and Figure 1 is in agreement with this with the highest growth attained in the oxygenated treatments (less than 2-fold increase in numbers). Due to the low overall growth level, no discernible trends between nutritional treatment and yeast growth could be established. It was likely that the absence of another nutritional factor present in normal brewers' wort was exerting a far greater effect. Treatments that received the protein-based stimulant appeared to flocculate more quickly and completely in this medium (Figure 1).

Figure 2 contains the fermentation rates established in all the treatments. The faster fermentation after 72 h for four treatments correlated to those that contained the stimulant either alone, or in combination, with the other nutrients. The most rapid (72 h) was the treatment with the stimulant, lipids, and oxygen although this difference was relatively small and disappeared after 72 h. Yeast requires the protein-based stimulant to alleviate CO₂ toxicity, the oxygen for all roles it fulfills, and the lipids for their nutritional value. This yeast generally synthesizes low unsaturated lipid levels when exposed to oxygen so the additional lipid was helpful in the lipid-free medium. We postulated that this difference would not be as noticeable in normal wort containing lipids.

The better performance of fermentations containing the protein-based stimulant led us to believe that wort particulate matter could play a role in stimulating fermentation performance. The fermentations containing the yeast stimulant gave rise to higher final alcohol concentrations that reflected the more rapid attenuation rates (Table 1). The FAN utilization profiles reflected these attenuation rates (Figure 3). The medium without supplementation contained yeast that performed very poorly in taking up assimilable or usable nitrogen. The provision of oxygen helped to promote growth and FAN utilization but CO₂ alleviation was required for full stimulation.

The SO₂ and acetaldehyde results indicated more differences between treatments. The simulated wort without supplements
The lowest acetaldehyde levels were found in treatments with all nutrients (1.0.2 mg/L). Malcorps et al (23) postulated how lipid decreased acetaldehyde concentrations by altering ester synthesis, however, lipids alone led to higher acetaldehyde concentrations (14.0 mg/L) as did any nutrient alone (14-16.6 mg/L). Any treatment that received added lipid demonstrated extremely low SO2 concentrations (2.4-2.9 mg/L). Exogenous lipid had a drastic effect on SO2 production. The reasons for this have been postulated by Dufour et al (13). The highest SO2 concentrations were found in the non-supplemented controls (15.2 mg/L). Those treatments that received the yeast stimulant alone, O2 alone or both these supplements showed intermediate but higher concentrations (11.9-13.4 mg/L).

**High Gravity Brewers’ Wort**

The yeast growth patterns achieved in production wort (16°P) in all the treatments are depicted in Figures 4A and 4B. All clear wort treatments achieved peak yeast counts after two days (4A) but cloudy wort treatments achieved peak counts only after three days (4B) with one exception. Yeast growth was delayed in the cloudy medium unless both oxygen and the stimulant were supplied. In this case, the growth rate was similar to that in clear wort; however, the overall growth level at peak count was reduced in the cloudy medium even if it received both oxygen and the protein supplement (4B). The possibility that the peak counts were missed due to the choice in sampling time was precluded due to other growth-related differences that demonstrated the lower counts were real effects (see later).

From Figures 4A and 4B, it is evident that the level of growth was reduced in the corresponding cloudy versus clear treatments. It is postulated that either the yeast cells became coated with the “cloudy” material thereby preventing optimal activity and growth by budding; or, that some inhibitory material to yeast was present in the cloudy wort but not in the clear wort. Alternatively, a nutrient (i.e. zinc) could have been more bioavailable in the clear versus the cloudy medium thus leading to stimulation.

The second two hypotheses could be partially dismissed as the provision of the protein supplement and oxygen restored the yeast growth rate in cloudy wort. Thus it is unlikely that an inhibitory compound was present in cloudy wort. However, the growth level was not restored to the same extent observed in clear wort containing both supplements so this possibility could not be completely negated. The bioavailability of zinc hypothesis (18) could be partially dismissed since the protein stimulant formulation contained appreciable amounts of zinc (6) but full restoration of growth required the dual presence of the stimulant and oxygen. The yeast crops harvested from cloudy wort treatments were visually assessed as being far dirtier than their clear wort counterparts. Therefore the occlusion of the yeast surface was thought to be the main contributor to the inhibitory effects observed in cloudy wort.

Yeast from treatments that received the protein stimulant flocculated more quickly in both wort types after nine days. This effect was similar to what we had observed in the simulated medium. Why this occurred was not clear. Fermentations did progress more quickly but not to an extent that would account for such disparate flocculation patterns. Early experiments investigating the mechanism of action of this protein stimulant showed that zinc in the formulation promoted flocculation. It is possible that the protein stimulant (or a component of it), once fermentation is complete, acts as a fining agent to cause more rapid and complete yeast flocculation. The ramifications of this could include several advantages. Better beer quality should
result as less yeast is carried over to autolyze later in the process, easier storage tank cleaning and better overall tank hygiene, no requirement for storage vessel fining agents if the stimulant addition rate is optimized; and, a better correlation between suspended yeast counts and residual extract making yeast harvesting (cropping) based on both measurements more practical for breweries.

Differences noted in the yeast growth patterns should be reflected in the overall fermentation rates. This was generally the case although the differences were not greatly pronounced (Figures 5A and 5B). Slightly slower overall fermentation took place in the cloudy wort. In both wort types the best overall fermentation performance was observed if both the protein stimulant and oxygen were supplied. This was particularly apparent in the cloudy wort where the overall yeast growth pattern was altered (Figure 4B). Of the two stimulants, it appeared that oxygen was more beneficial than the protein stimulant to both yeast growth (especially Figure 4B) and attenuation (particularly Figure 5A early in the fermentation). However, the two acted synergistically to enhance fermentation performance (Figures 5A and 5B).

Since the fermentation rate was affected to a lesser degree than yeast growth in cloudy versus clear wort, one may argue that the cells in the cloudy wort demonstrated a higher fermentation capacity on a per cell basis than did cells from the clear wort. This is very difficult to prove or disprove. The end alcohol percentages were similar with treatments demonstrating faster initial fermentation also showing slightly higher ethanol concentrations (Table 2). Thus higher growth did not significantly compromise end product concentrations although yields on a per cell basis would have been lower. It is well accepted in the alcohol industry (fuel or beverage) that faster attenuation leads to higher ethanol concentrations and less by-product formation (27,36).

Figures 6A and 6B contain the assimilable or usable nitrogen uptake pattern as measured by free amino nitrogen (FAN). It was expected that the cloudy wort FAN concentration would drop slower than that in the clear wort due to the yeast growth pattern differences. The uptake of FAN and the yeast growth rate and extent are linked phenomena (11,21,26). This did occur with slower initial FAN uptake in the cloudy versus clear worts except for the cloudy treatments that received both oxygen and the protein stimulant. The yeast cells in this treatment grew faster than cells in any of the other cloudy wort treatments (Figure 4B).

A more surprising observation was the great difference between residual FAN levels in the different treatments and between the two wort types. In most cases this could be attributed to the greater level of growth in the clear wort treatments. It is known that residual FAN can lead to the formation of staling compounds (25) and thus, the end concentration should be minimized through optimizing yeast growth and activity.

Table 3 contains the concentrations of diacetyl and 2,3-pentanedione at the end of fermentation. Cloudy or clear wort containing no stimulants demonstrated the highest concentrations and worts containing both supplements contained the lowest end concentrations. Wort clarity did not affect the final vicinal diketone concentrations. The protein supplement appeared to be slightly more stimulatory to VDK removal than oxygen although both were required for acceptable removal. Dimethyl sulfide (DMS) concentrations were similar in all the treatments (data not shown).
Table III
Final VDK Concentrations in the Wort Fermentations

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>DIACETYL (µg/L)</th>
<th>PENTANEDIONE (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clear, O₂</td>
<td>68</td>
<td>46</td>
</tr>
<tr>
<td>Clear, Protein Stimulant</td>
<td>65</td>
<td>37</td>
</tr>
<tr>
<td>Clear, Both Additions</td>
<td>23</td>
<td>13</td>
</tr>
<tr>
<td>Clear, No Additions</td>
<td>129</td>
<td>102</td>
</tr>
<tr>
<td>Cloudy, O₂</td>
<td>76</td>
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<tr>
<td>Cloudy, Protein Stimulant</td>
<td>68</td>
<td>49</td>
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<td>31</td>
<td>13</td>
</tr>
<tr>
<td>Cloudy, No Additions</td>
<td>143</td>
<td>114</td>
</tr>
</tbody>
</table>

Results are means of duplicate fermentations, samples were taken from each fermentation and analyzed, the values are the means of the two determinations.

Table IV
Final Acetaldehyde and SO₂ Concentrations in the Wort Fermentations

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>ACETALDEHYDE</th>
<th>SO₂ (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(mg/L)</td>
<td>Total</td>
</tr>
<tr>
<td>Clear, O₂</td>
<td>22.7</td>
<td>13.0</td>
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<tr>
<td>Clear, Protein Stimulant</td>
<td>21.9</td>
<td>20.4</td>
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<td>Cloudy, O₂</td>
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<td>21.7</td>
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<td>9.0</td>
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<tr>
<td>Cloudy, No Additions</td>
<td>24.1</td>
<td>18.1</td>
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</table>

¹. Amount of unbound SO₂. The difference between the total and free concentrations is the amount of bound SO₂.

The results are the means of duplicate fermentations, samples were taken from each fermentation and analyzed, the results represent the means of the two analyses.

Acetaldehyde and SO₂ concentrations at the end of fermentation are contained in Table 4. The only discernible trend was that both stimulants were required to reduce the end acetaldehyde concentrations in either wort. The cloudy wort containing only oxygen also performed well in this regard. The clarity of the wort exerted no effects on acetaldehyde production. The failure to provide adequate nutrients and/or stimulants translated to a stressful condition for the yeast. We have previously demonstrated that yeast stress leads to higher acetaldehyde and SO₂ concentrations (21,26,29,30,31). Final SO₂ concentrations indicated yeast stress unless oxygen was supplied to the wort either alone or in combination with the yeast stimulant. Oxygen is recognized as a major regulator of sulfur dioxide production by yeast during fermentation (13,28). The alleviation of CO₂ toxicity using the protein stimulant could not reduce SO₂ production. There was no relationship between SO₂ production and wort clarity. The effect of exogenous lipids noted in the simulated wort was not repeatable in actual wort and the lipids in the cloudy wort appeared to have no effect in this regard.

Another possible indication of yeast stress is the beer protease concentration in each treatment. These results were low in all the treatments when measured on Day 6 (data not shown). Slightly higher activity was noted in cloudy wort containing the protein stimulant and oxygen. Thus the restoration of the yeast growth rate in this treatment (Fig. 4B) was accompanied by signs of overall yeast stress. No additional trends could be established in the other treatments 6 h, 6 days or 13 days after inoculation (data not shown).

The results from the laboratory experiments allowed us to reach some important conclusions. Cloudy wort led to poorer yeast growth, slower attenuation, and higher residual FAN levels. Some of these inhibitory effects could be overcome by the
provision of adequate yeast stimulants but the overall fermentation performance was much more reliant on constant oxygenation and the alleviation of CO₂ toxicity. The former is often difficult in breweries due to the non-optimized wort oxygenation systems. The latter is also problematic if the stimulant is added either to yeast slurry which is not efficiently mixed prior to pitching or if added to hot wort, removal in the whirlpool can occur. The most efficient addition point in terms of mixing is the hot side of the wort paraflow and if the protein supplement is added here, consistent effective concentrations result.

The formation or removal of flavor active substances showed no real correlation to the clarity of the wort per se. Their concentrations reflected the overall fermentation rates. The failure to provide adequate oxygen or alleviate CO₂ toxicity led to much larger variations in these substances than lack of wort clarity. In simulated wort, added lipids greatly depressed SO₂ production. In production wort, cloudy wort lipids were ineffective in this regard. The effects in the simulated medium could have been largely due to the added ergosterol which is more critical to brewing yeast nutrition (3,11,26). The laboratory experiments proved that cloudy wort does not provide nutritional value to this yeast strain or eliminate CO₂ toxicity due to its particulate content. In the laboratory, no cloudy wort treatment performed better than its clear wort counterpart.

Brewery Trials

If it is recommended that cloudy worts be avoided, what are the practical means to achieve this? Wort filtration can remove turbidity. For years, Stroh Brewery filtered cold wort through diatomaceous earth powder filters to obtain initial brilliance (34). However, they found this expensive in labor and disposal costs and found that adding a portion of unfiltered wort improved their fermentation performance. This was later traced to particulate matter acting as CO₂ nucleation sites (34) in the absence of an alternative stimulant such as the one used in this research.

Unless the whirlpool is known to be operating inefficiently and performance here has not been maximized, there is little room for improvement in this area. Whirlpools are not designed to remove “cloudy” components. One can, however, alter the “cloudy” material in a manner that allows the whirlpool to more efficiently remove it. So-called “kettle or copper fining” techniques do this. For instance, the use of carrageenan in the kettle leads to aggregation of the turbidity components. They are then more efficiently removed during hot break separation or alternatively, in the whirlpool. Commercial scale trials that employed carrageenan finings were conducted and the following results were achieved.

Figure 7 depicts the yeast growth patterns in the trial (clarified with kettle finings) versus the control worts for two separate tests. As was the case with the laboratory-scale work, yeast in clear wort grew more quickly than that in cloudy wort. The slower growth rate in cloudy wort led to a slower fermentation that delayed flocculation until much later. Approximately the same maximal yeast count was achieved in all the fermentations indicating that no inhibitory substance that reduced the yeast growth level was present in cloudy worts. The use of kettle finings had no impact on the wort FAN concentrations (data not shown).

It is evident from the yeast count results in Figure 7 that yeast could be harvested earlier from the clear wort fermentations. This has the added advantage of not allowing the yeast to deteriorate in the bottom of the vessel which is a highly unfavorable environment for the cells. In the cloudy wort fermentations, cropping was delayed since yeast flocculation was the only means of separating yeast from beer available in this brewery. Some cells would have been at the bottom of the fermenter for several days prior to yeast cropping. This differential flocculation pattern can be expected to impact on the vitality of the harvested yeast and the subsequent quality of beers produced from it.

Table 5 contains the pH values throughout fermentation in representative trial versus control beers. As would be expected, the pH drop was more rapid in the trial beer reflecting the more rapid growth and fermentation rates. Organic acid production as
more yeast autolysis occurred in the control brews due to the colloidal unstable (25). Low end pH values compared to the controls. It is possible that deduced from the data in Table 6. The production of the buffering proteins were removed via the kettle fining process to increase as CO₂ escaped solution. The trial beers demonstrated as the fermentation rate slowed, the pH was sometime observed well as more dissolved CO₂ in solution promoted these effects. As the fermentation rate slowed, the pH was sometime observed to increase as CO₂ escaped solution. The trial beers demonstrated low end pH values compared to the controls. It is possible that buffering proteins were removed via the kettle fining process accounting for some of this pH difference. It is also possible that more yeast autolysis occurred in the control brews due to the differential flocculation pattern (Figure 7). This aspect of kettle fining needs more attention as low pH beers are often flavor and colloidal unstable (25).

The pattern of diacetyl production and removal can be deduced from the data in Table 6. The production of the precursor of this compound was related to the pattern of yeast growth whilst its conversion to diacetyl was pH-related (lower pH favoring the reaction). This chemical conversion is the rate-lim-
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