Yeast Handling Studies. I. Agitation of Stored Pitching Yeast

Robert McCaig and Dirk S. Bendiak, *Molson Breweries of Canada Ltd., 2486 Dunwin Drive, Mississauga, Ontario, Canada L5L 1J9*

ABSTRACT

The effects of agitation on stored production yeast were studied to improve yeast handling procedures. Yeast stored 5 days at 1°C with constant agitation decreased in viability by 25% and in glycogen, 85%. The yeast cells were granular, and slurry cell numbers were reduced by 15% owing to mechanical breakage caused by the agitation. Yeast stored with no agitation (still) under beer or water decreased in viability only 8% and in glycogen content, 12%. Yeast stored with a 2-hr agitation per day (a time long enough to maintain slurry homogeneity) did not differ significantly from yeast stored still. When the stored yeasts were pitched into high gravity worts (15.1° P), yeast stored still and yeast stored with minimal agitation produced similar results in terms of attenuation rate, final attenuation, yeast in suspension, pH, alcohol production, final diacetyl concentration, and dimethyl sulfide concentration. Significant differences were noted in fermentations pitched with yeast constantly stirred during storage. Attenuation was slower, final attenuation was higher, yeast in suspension was lower and slower to increase in numbers, and the concentrations of diacetyl and dimethyl sulfide were much higher than the aforementioned types.

Key words: Agitation, Attenuation, Beer quality, Yeast viability

In the brewing industry, maintenance of a product consistent in quality and taste depends essentially on the stability of the biochemical properties of the brewery yeast. Degeneration of the yeast occurs during fermentation (12) and during storage before repitching (7,9). The sometimes drastic changes in yeast biochemistry that occur during storage are inherent in the yeast (12). During storage the yeast is presented with a static nutrient-deficient medium. Under storage conditions, the yeast utilizes endogenous reserves to produce energy needed for maintaining essential cellular metabolic functions. The changes that can occur are manifested in subsequent fermentations as a slowing rate of fermentation and a decrease in the final attenuation. The resultant beer can then contain fermentable sugars that would normally have been utilized and elevated levels of compounds that are normally assimilated when all the fermentable sugars are metabolized (1). The origin of the degeneration is most likely to be found by examining variations that occur in yeast as a response to changes in environment.

At present the most popular form of yeast storage before repitching seems to be under beer or water (3,16) in a nonagitated state (19). In this procedure, loss of viability of the stored yeast is minimal if the storage temperature is kept low (9).

Storage of production yeast with constant agitation is another popular yeast handling technique (3,16). Yeast is constantly stirred to maintain a slurry homogeneous in both consistency and temperature. A homogeneous slurry is important when yeast is pitched into wort by volume rather than by weight. Addition of oxygen to stored yeast by agitation has been suggested as a method of yeast handling that may allow a brewer to forego wort aeration or oxygenation and may result in faster fermentation (8,10). This study, therefore, deals with the agitation of stored pitching yeast and the subsequent behavior of the yeast when pitched.

MATERIALS AND METHODS

Storage

The yeast used was production ale yeast (*Saccharomyces cerevisiae*) collected fresh from a production fermenter. Yeast was stored in 600-ml aliquots in sterile 1 L flasks. Agitation was achieved with sterile stir bars and a Corning PC351 magnetic stirrer (Corning Glassworks, Corning, NY) set at medium speed (a slight vortex formed at this speed). By tachometer readings, this speed was equal to 300 rotations per minute in water. The temperature of storage in these experiments was 1°C and the storage time was 5 days. All experimental storages were conducted in triplicate.

Daily samples were removed from the stored yeast to assess viability and glycogen content. Samples from yeast agitated only 2 hr/day were removed after the agitation period.

Fermentation

After storage, the yeast was acid washed for 2 hr at pH 2.2 with 8.5% phosphoric acid (standard washing procedure at Molson). The yeast was then pitched into aseptically collected high gravity production wort of specific gravity 15.1° Plato. Based on a packed cell volume, pitching levels of 15×10^6 cells per milliliter were obtained. Fermentations were conducted in triplicate at 20°C in vessels previously described (13,14). The fermentations were conducted without stirring as in production fermentations.

Daily samples were removed to assess yeast in suspension, specific gravity, pH, alcohol, and diacetyl concentration. Wort and end fermented beer were examined to determine levels of dimethyl sulfide (DMS) and its precursor (DMSP).

Analyses

Viability was assessed by spread plating an appropriate dilution of the sample in triplicate onto wort agar. Counts were compared with original hemacytometer counts to determine actual viability. Budding yeasts were counted as one colony forming unit only.

Glycogen was determined using the rapid method of Quain and Tubb (20). This procedure was standardized with the enzymatic method of glycogen determination of Quain (17).

Respiratory deficient yeasts were determined by the method of Ogur et al (15).

Pitching was accomplished by determining the packed cell volume of the prestorage slurry. In this method, hemacytometer counts were correlated with the amount of sediment collected from a standard aliquot of sample that underwent a standardized centrifugation in a standardized tube. A standard curve was obtained for the ale yeast slurry used, and from this curve, yeast in suspension was determined by centrifugation of an aliquot of the fermentation.

Specific gravity and alcohol concentration were determined using a DMA55 Densitometer (Anton Paar, Graz, Austria) in tandem with a flow-through immersion refractometer (Carl Ziess Ltd., Don Mills, Canada). A digital 110 pH meter (Corning Glassworks, Corning, NY) was used to determine pH.

DMS and DMSP were determined by the methods of Hysert et al (5,6). Diacetyl was measured by the ASBC Vicinal Diketone Subcommittee (1983–84) headspace gas chromatographic method, using an Hewlett Packard 5840A gas chromatograph equipped with an electron capture detector.

RESULTS AND DISCUSSION

The reason for the widespread use of still storage of yeast was obvious when the results of yeast viability and glycogen content during still storage under beer (SS yeast) were examined (Figs. 1 and 2). Over the 5-day period of storage, viability (Fig. 1) for SS yeast decreased from 96 to 89.3%, a minimal reduction. Similarly, glycogen content of SS yeast decreased 12% from 342 to 265 mg/L of slurry. Glycogen content decreased from an initial level of 32% of the yeast dry cell weight to 28%. In a similar study (19), glycogen

content of SS yeast decreased from 47 to 30% on a dry weight basis during a 1-week storage. The reduction in glycogen was due to the use of stored cellular glycogen as an energy source in maintaining cellular functions (18,19).

Similar reductions in yeast viability and glycogen content were recorded for yeast stored still under water; however, a slight decrease in slurry cell numbers attributable to cell rupture caused by osmotic imbalances was noted. Replacing beer with water as a suspending medium for the yeast dramatically altered the cellular osmotic balances. Cellular swelling, resulting from water flowing into the cells, caused weaker cells to rupture. Because results for yeast stored under beer or water were similar, only the former will be reported.

Subsequent investigations showed that SS yeast could be maintained as long as 3 weeks without losses in viability (15%) and glycogen content (35%) significant enough to affect fermentations subsequently pitched with this stored yeast. These results reinforced Quain and Tubb's previous similar results (19).

Yeast stored with constant agitation (CA yeast) decreased in viability twice as fast as the SS yeast rate (Fig. 1) during the first 3 days and four times as fast during the last 2 days of storage. Final yeast viability for CA yeast was 70%, a level 21% lower than SS yeast after an equivalent 5 days of storage. This extreme reduction in viability was reflected in a similar pattern of reduction for CA yeast glycogen content (Fig. 2). Apparently, oxygen provided to the yeast by the mechanical action of agitation accelerated the metabolic processes of the yeast cell. This required the expenditure of larger amounts of energy for cell maintenance (19). The source of this required energy was stored cellular glycogen. Weak, damaged yeast, or those yeast cells initially low in glycogen, would have depleted storage reserves sooner. With no source of energy, cell death would occur much faster, resulting in lower slurry viabilities. Interestingly, when viability was plotted against glycogen content, a linear regression analysis revealed a direct linear correlation between the two (Fig. 3). The coefficient of correlation obtained was 0.987 and the equation of the line of regression was $Y = -75.5 + 3.3X$. The line intercepts the X-axis, indicating that yeast remains viable while possessing no internal glycogen reserves (Fig. 3). This condition exists 18–24 hr into a fermentation when all yeast glycogen reserves have been catabolized. This analysis showed an intimate link between cell viability and cell glycogen content.

Glycogen levels in CA yeast decreased steadily during the 5-day storage (Fig. 2). Final values decreased to 51 ml/L of slurry, a reduction of 86% from the original levels before storage. In a similar study (19), yeast stored at 2° C with agitation decreased 20% in glycogen levels in 2 days, a figure comparable to our results. Glycogen in the presence of oxygen, is catabolized by oxygenases (18) to form sterols required by the yeast to commence the fermentative phase. Jakobsen (7) suggested that preoxygenation of yeast slurries instead of the wort would decrease the initial lag phase. Quain and Tubb (19) discerned that when 200 mg of glycogen was catabolized by yeast in the presence of oxygen, 6.4 ± 1 mg of sterol was formed. This reaction occurred in wort, which is rich in carbohydrate and nitrogen, whereas our preoxygenation occurred in nutrient-deficient conditions. Therefore, all glycogen catabolized was not metabolized into sterol. If the glycogen dissimilated from CA yeast during storage was changed to sterol, the concentration of sterols in the yeast at pitching should have been 9.6 mg/L of slurry, a concentration sufficient to negate the need for wort oxygenation and to shorten the initial lag phase after pitching (10). In subsequent fermentations, CA yeast was not high in sterols after storage.

Addition of oxygen by agitation may have caused additional loss of viability by oxygen toxicity (4). The partial pressure of oxygen required to initiate metabolic derangements of microorganisms is, in many cases, not much greater than the partial pressures at which the microorganisms usually grow. The precise mechanism of microbial oxygen toxicity is not known, but multiple cell sites are believed to be affected (4).

Also noted in CA yeast after storage was the large percentage of cell breakage that occurred during storage. Using the hemacytometer, we estimated that in addition to a 26% loss in cell viability, a further 15% of the original yeast cell numbers was lost through mechanical breakage.

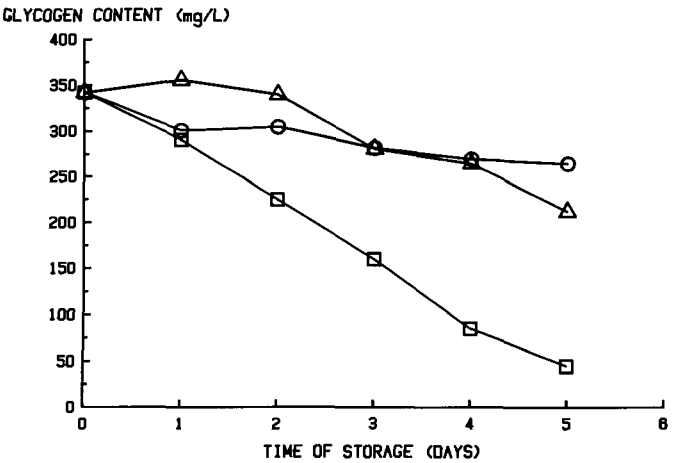

Fig. 2. Glycogen content (mg/L) of ale yeast stored. O = Still under beer (SS), □ = constantly agitated (CA), Δ = with 2 hr agitation per day (2A).

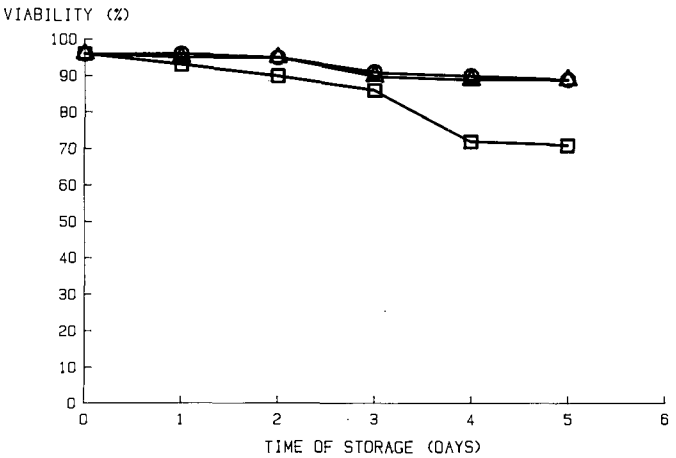

Fig. 1. Viability (%) of ale yeast stored. O = Still under beer (SS), □ = constantly agitated (CA), Δ = with 2 hr agitation per day (2A).

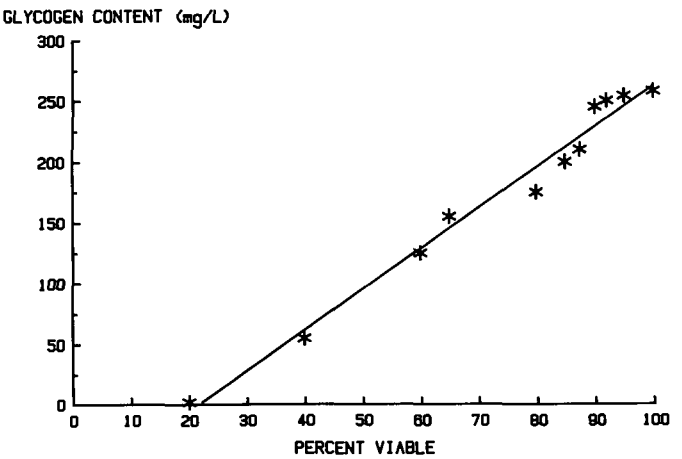

Fig. 3. Linear regression analysis of glycogen content and viability in ale yeast stored 5 days.

A significant increase in respiratory deficient yeast was noted after 5 days of CA yeast storage (Table I). Respiratory deficient mutants increased from 1 to 15% of the population. In SS yeast, the percentage of respiratory deficient mutants increased from 1 to 1.5% during storage. It is believed that the smaller size of respiratory deficient mutants better protected these cells from mechanical breakage by the agitation. This phenomenon has been noted previously with centrifuged yeast (*unpublished data*).

To satisfy brewers who require a homogeneous slurry, use of limited agitation of the slurry was investigated. An agitation period of 2 continuous hours per day during the storage period adequately maintained slurry homogeneity. Yeast stored this way (2A yeast) did not experience significant reduction of viability (Fig. 1) or glycogen content (Fig. 2). When yeast was similarly stored with agitation longer than 2–4 hr/day, significant changes in glycogen content and viability occurred. Viability of 2A yeast during storage followed the pattern and level recorded by SS yeast during storage (Fig. 1).

The glycogen content (Fig. 2) of 2A yeast did increase slightly the first day in all nine replicate analyses and fermentations. No adequate hypothesis could be given for this phenomenon. Glycogen levels should have decreased by 10–20% after the first day of storage as levels did in SS yeast and CA yeast. Oxygen made available to the cells by the 2-hr agitation should have depleted glycogen reserves in 2A yeast faster than in SS yeast but slower than in CA yeast. The normal pattern of glycogen catabolism during storage was exhibited in 2A yeast after the first day of storage. The rate of glycogen decline was equal to the rate recorded by SS yeast during storage (Fig. 2). Final levels of glycogen in 2A yeast were 20% lower than in SS yeast, an amount equal to one additional day of storage of SS yeast.

No significant increases in the percent of respiratory deficient yeast occurred in the 2A yeast during storage (Table I). Results were similar to the slight increases seen during SS yeast storage.

After storage, the yeast was acid washed and then pitched into duplicate fermentations of aseptically collected production wort. All yeast storage types were pitched by volume calculated by examining the packed cell concentration of the stored SS yeast. No allowances were made for reduced yeast viability or reduced cell numbers (due to mechanical breakage of cells).

Fermentations pitched with SS yeast and those pitched with 2A yeast showed similar attenuations (Fig. 4). In 2A yeast fermentations, the rate of attenuation was slower at the midpoint of the fermentation (between 48 and 85 hr after pitching). As a result, the final specific gravity of the fermentations pitched with 2A yeast was higher (2.45° P) than that of fermentations pitched with SS yeast (2.3° P). The reason for these slight differences in attenuation was attributed to lower numbers of yeast in suspension in 2A yeast fermentations (Fig. 5), although the typical pattern of yeast cell growth during a fermentation was noted.

TABLE I
Respiratory Deficiency of Stored Yeast, and Final pH, Dimethyl Sulfide (DMS), and Dimethyl Sulfide Precursor (DMSP) Levels in the Trial Fermentations Conducted with the Yeast After Storage

Method	Yeast Respiratory Deficiency After Storage (%)	Trial Fermentations		
		Final pH	DMS (μg/L)	DMSP (μg/L)
Still (SS yeast)	1.5	4.03	69	102
Agitated 2 hr/day (2A yeast)	1.4	4.03	86	109
Agitated Continuously (CA yeast)	15	4.52	352	122

Although the production of ethanol and degree of attenuation are directly related in normal yeast, changes in yeast metabolism could occur during storage. These changes could result in variations in the quantity of primary and secondary metabolites produced. Production of ethanol by SS yeast and 2A yeast during fermentation was nearly identical (Fig. 6). The pattern of ethanol production in relation to attenuation was normal. The most rapid increase in ethanol production correlated to the most rapid period of attenuation.

Final pH values for both SS yeast and 2A yeast fermentations were similar (Table I). Fermentations pitched with SS yeast finished at pH 4.05 and those pitched with 2A yeast finished at pH 4.03, an insignificant difference.

Subtle differences in produced diacetyl were seen in the trial fermentations pitched with SS yeast or 2A yeast (Fig. 7). Although final diacetyl concentrations were the same (0.085 mg/L), the peak concentration of diacetyl, which occurred 40 hr after pitching, was elevated in 2A yeast fermentations by 18%. Reduction of viable yeast in suspension in 2A yeast fermentations (Fig. 5) was probably the reason for elevated peak in diacetyl concentration. Weak or damaged cells caused additional leakage of diacetyl from internal cellular pools (21). In addition, mechanical agitation or oxygen toxicity (4) may have caused shifts in metabolic pathways in the yeast, perhaps favoring production of excess diacetyl. Although reduced in numbers, 2A yeast was able to reassimilate the excess diacetyl at a rate faster than did SS yeast (Fig. 7).

Levels of DMS (Table I) in the final beer were 25% higher in fermentations pitched with 2A yeast than in those pitched with SS yeast. The DMS level in both final beers, however, was lower than the original wort DMS level of 112 μg/L. Less DMS was removed by the 2A yeast because the fermentations pitched with 2A yeast were less vigorous owing to the addition of less yeast at pitching (lower viabilities of 2A yeast) or yeast damage caused by the mechanical action of stirring. Oxygen toxicity may have caused metabolic changes (4) in 2A yeast during storage. Beer DMS levels for both SS yeast and 2A yeast were not so high as to cause flavor defects. DMSP levels (Table I) decreased approximately 50% in the fermentations pitched with either 2A yeast or SS yeast. Original wort DMSP levels were 221 μg/L.

Beers produced by SS yeast or 2A yeast in our trial fermenters were considered to be well within specification for all characteristics examined, according to normal ale fermentations conducted in our trial fermenters.

Examination of fermentations pitched with CA yeast revealed enormous differences in all investigated characteristics compared with either SS yeast or 2A yeast fermentations. We commented

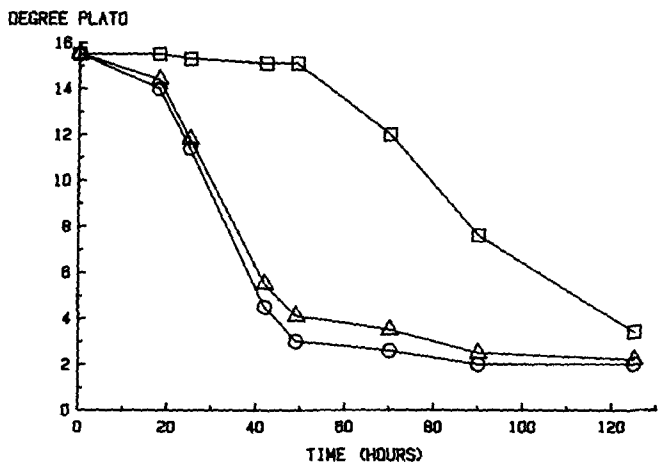

Fig. 4. Specific gravity (° P) of trial fermentations pitched with ale yeast stored 5 days. O = Still under beer (SS), □ = constantly agitated (CA), Δ = with 2 hr agitation per day (2A).

earlier that CA yeast may experience a shorter lag period. This related to the presence of sterols produced by the catabolism of glycogen in the presence of oxygen introduced by the constant agitation. A rapid decline in specific gravity should have occurred immediately on pitching, but this did not occur. Instead, the fermentations pitched with CA yeast exhibited no change in specific gravity for the first 50 hr after pitching (Fig. 4). The subsequent rate of attenuation was less than half the rate recorded in fermentations pitched with SS yeast or 2A yeast. The final attenuation was not complete until 48 hr after both the SS yeast and 2A yeast fermentations had completely attenuated. The final specific gravity of CA yeast pitched fermentations was 3.7° P, a value 1.4° P higher than fermentations pitched with SS yeast or 2A yeast (Fig. 4).

The modification noted in attenuation of CA yeast fermentations was directly related to the numbers of yeast in suspension (Fig. 5). The yeast in suspension curve recorded for CA yeast fermentations was vastly different from the usual curve for normal ale fermentations. Instead of an initial period of rapid increase in yeast cell numbers immediately after pitching, a period of decrease in yeast cells in suspension was experienced. Yeast numbers dropped from 15×10^6 cells per milliliter at pitching to 4.2×10^6 cells per milliliter 42 hr after pitching. Cells in suspension then began to rise slowly until a peak level of 15.6×10^6 cells per milliliter was reached 70 hr after pitching. The peak was substantially lower than the peaks recorded in the fermentations pitched with SS yeast or 2A yeast (Fig. 5). The reason for the initial decrease in yeast in suspension was thought to be the result of low viability and increased cell breakage. Pitching by packed cell volume with a yeast of 70% viability and an additional 15% broken cells resulted in only 8.25×10^6 healthy colony forming units per milliliter when a packed cell volume measurement indicated 14×10^6 cells per milliliter. At 20 hr after pitching, the yeast in suspension was reduced to the expected viable pitch rate of 8.25×10^6 cells per milliliter, but the drop in cell numbers in suspension increased, although values had been expected to begin to increase normally from that level. This indicated that another factor was to be considered. Cells damaged by mechanical agitation, oxygen toxicity, or low glycogen reserves may not have survived the high osmotic and glycolytic pressures exerted by the high gravity wort environment (2). Subsequent investigations revealed that this additional decrease was negated when the yeasts were suspended in a wort of lower specific gravity.

Production of alcohol in CA yeast fermentations (Fig. 6) was altered drastically from curves recorded in the other stored yeasts (SS yeast, 2A yeast). The rate of alcohol production was considerably reduced, as was the total amount of alcohol produced. Final levels of alcohol were reduced 21% from values recovered in SS yeast or 2A yeast pitched fermentations.

The final pH of CA yeast fermentations was much higher than for the other two yeast storage types of fermentations (Table I). Because the fermentations pitched with CA yeast did not attenuate fully, a higher pH was expected.

Diacetyl production in CA yeast fermentations changed dramatically (Fig. 7). A lag in diacetyl production equal to the lag experienced in attenuation (Fig. 4) and yeast in suspension (Fig. 5) was recorded. Production of diacetyl increased slowly, commencing 40 hr after pitching. In addition, diacetyl produced was not reassimilated by the yeast toward the end of fermentation as is normal in production fermentations (19). The constant agitation of CA yeast appeared to have upset the normal metabolic balance. Poor viability of pitched yeast and an unusually long lag phase alone were not responsible for the apparent loss of CA yeast ability to reassimilate diacetyl during late fermentation. This increase in diacetyl production in fermentations pitched with preagitated yeast has been noted previously (11).

Final levels of DMS (Table I) in CA yeast fermentations were five times the levels reported in either SS yeast or 2A yeast pitched fermentations. In addition, DMS levels in CA yeast final beer were three times higher than in the original wort. The CA yeast fermentations were very slow; therefore, we assumed that little or

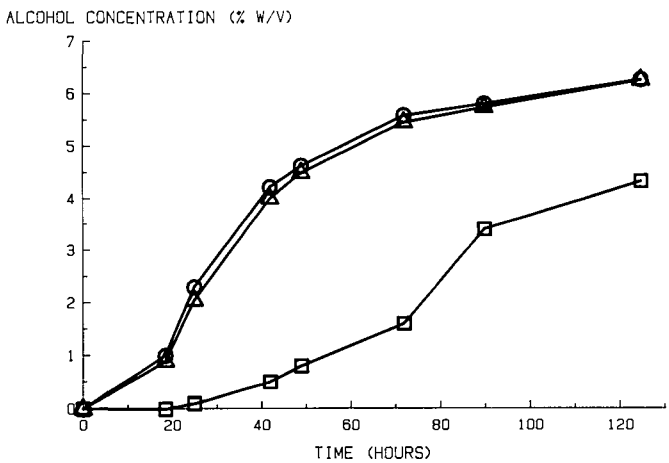

Fig 6. Alcohol concentration (% w/v) in trial fermentations pitched with ale yeast stored 5 days. O = Still under beer (SS), □ = constantly agitated (CA), Δ = with 2 hr agitation per day (2A).

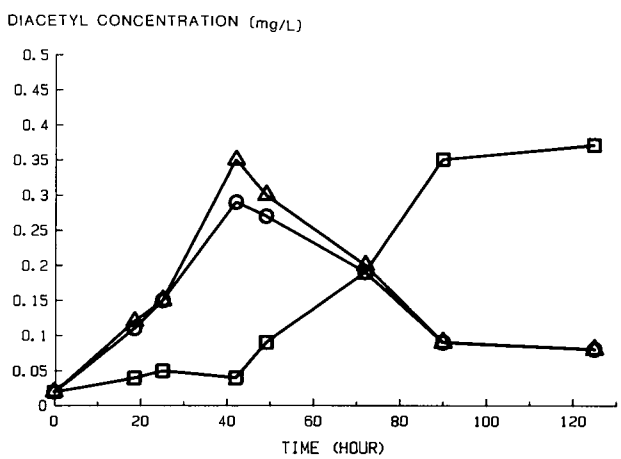

Fig. 5. Number of yeast in suspension (colony forming unit/ml) in trial fermentations pitched with ale yeast stored 5 days. O = Still under beer (SS), □ = constantly agitated (CA), Δ = with 2 hr agitation per day (2A).

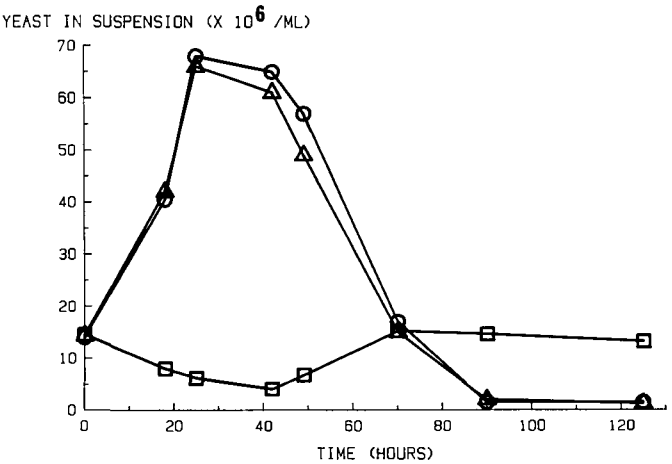

Fig. 7. Diacetyl concentration (mg/ml) in trial fermentations pitched with ale yeast stored 5 days. O = Still under beer (SS), □ = constantly agitated (CA), Δ = with 2 hr agitation per day (2A).

no DMS was removed by the normal CO_2 scrubbing process. However, an additional 240 $\mu g/L$ of DMS in the final beer indicated that DMS was produced during the CA yeast fermentations. Results of microbiological testing revealed no wild yeast or bacterial contamination. We therefore hypothesized that the yeast produced the extra DMS. Metabolic rearrangements caused by oxygen toxicity or mechanical agitation could have caused the yeast to produce DMS or to lose cell membrane integrity, in turn causing the yeast to leak to the surrounding medium internal pools of stored DMS. DMSP levels were similar to those reported for the other two yeast types (Table I).

CONCLUSIONS

When any pitching yeast is stored for longer than 72 hr with constant agitation, the slurry will possess lower cell numbers, lower viabilities, and lower glycogen content. Fermentations subsequently pitched with a yeast slurry stored with agitation will have longer lag phases, lower numbers of yeast in suspension, poorer rates of attenuation and final attenuation, higher diacetyl levels, higher DMS levels, and lower alcohol yields.

If yeast is to be stored for a long period of time before being pitched, ideally it should be stored still under beer. If a brewer absolutely requires a completely homogeneous slurry, the agitation period should be no more than 2 hr/day.

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