Yeast Handling Studies. II. Temperature of Storage of Pitching Yeast

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

ABSTRACT

The methods employed in handling production yeast before pitching are important. Fermentations pitched with improperly handled yeast could experience problems such as hanging fermentations or poor attenuation or both. The temperature of yeast storage is a major factor when considering handling procedures. Yeast stored 6 days at 1°C showed a 5% drop in glycogen content and a 7% drop in viability. The same yeast stored at 5°C decreased 22% in glycogen content and 14% in viability. At storage temperatures of 10, 15, 20, and 25°C, significant reductions in glycogen content (up to 79%) and viability (up to 68%) occurred. Fermentations pitched with yeast stored at 1 and 5°C showed little difference in numbers of yeast in suspension; rate of attenuation; final attenuation; diacetyl, alcohol, and dimethyl sulfide concentrations; or final pH. Fermentations pitched with yeast stored at 10°C exhibited higher final diacetyl and dimethyl sulfide concentrations. The fermentations pitched with yeast stored at 15, 20, and 25°C did not ferment normally; rate and extent of attenuation were poor; the number of yeast in suspension was abnormal; subsequent yeast crops were less; diacetyl, alcohol, and dimethyl sulfide concentrations were less; and final pH was much higher.

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

Continual use of pure healthy yeast cultures eliminates one major source of variation in the brewing process. Normal attenuations in terms of rate and final values are achieved, and flavor variations between brews are minimized. As previously discussed (11), changes in inherent yeast makeup occur (10), causing degeneration of the yeast during storage (8,9). The effect of agitation was an acceleration of this degeneration (11).

Although the influence of temperature on yeast growth has been extensively investigated (6), the effect on storage of brewing yeast has not been adequately studied. Yeast in storage is in a catabolic state whose level of activity is largely temperature-dependent (6). Temperature is an environmental factor, any variation of which is liable to exert a profound effect on the yeast and hence on the subsequent fermentation. Autolysis begins at moderate temperatures of yeast storage (12). It occurs when yeast stored in a nonfermentable liquid begins to metabolize stored, internal supplies of energy-producing compounds (12). When those are exhausted, the yeast cell begins to degrade vital cell components and organelles to produce needed energy (12). The products of autolysis can provide nutrients for contaminating bacteria and can also exert an adverse effect on the flavor character of the final beer (2). Roessler (13) suggested that the temperature of yeast storage be no higher than 3°C, because above that temperature, yeast fermenting power will be impaired and subsequent fermentation will exhibit longer lag phases, reduced wort pH, and poor final attenuation. At temperatures between 10 and 20°C, a phenomenon called "autofermentation" can occur during storage (7). Reserve carbohydrates (particularly glycogen and trehalose) are fermented to produce ethanol and carbon dioxide. This phenomenon can cause a more rapid loss in slurry viability than autolysis.

The temperature at which yeast is stored is of vital importance in maintaining a viable, stable yeast culture that will remain consistent in terms of brewing characteristics (e.g., rate of attenuation, flocculation). It was felt that temperatures of 1, 5, 10, and 15°C were representative of yeast storage temperatures in modern yeasting practices (13). Results of yeast stored at 20 and 25°C are included in an attempt to determine the possible effects of major breakdown in cooling during yeast storage.

MATERIALS AND METHODS

Storage

The storage temperatures investigated were 1, 5, 10, 15, 20, and 25°C. Yeast samples were stored still under beer for 6 days.

Analysis

All analyses were conducted as described previously (11).

RESULTS AND DISCUSSION

In examining the daily viabilities of the yeast during storage at the six test temperatures (Fig. 1), we grouped the resulting data into one of two response groups based on the rate of decrease in viability or the final viability. The first response group was characterized by a slow rate of decrease in viability during storage and final viabilities ranging from 10 to 22% less than the original yeast viability before storage. In addition, the decrease in yeast viability during the first 2 days of storage was similar regardless of the temperature of storage. Yeasts stored at 1, 5, and 10°C were placed in this response group. Final viabilities for yeast stored at 1, 5, and 10°C were 85, 82.5, and 72.5%, respectively.

Yeast stored at 15, 20, and 25°C formed the second group, which was characterized by a much faster rate of decrease in viability (four times that of the other group) and final viabilities ranging from 55 to 68% less than the original yeast slurry. In addition, differences in the viability between yeast stored at these three temperatures could be discerned after only 1 day of storage (Fig. 1). Final viabilities were 42.5, 35, and 30% after storage 6 days at temperatures of 15, 20, and 25°C, respectively.

It was also apparent from the data in Figure 1 that at storage temperatures higher than 10°C, a dramatic increase in the loss of cell viability occurred almost immediately. As the temperature of storage increases, the energy required for yeast cell maintenance increases (12). Increasing temperature accelerates the metabolism in the stored yeast, which in turn results in the utilization of internal reserves stored in each yeast cell. An indication of the dramatic increase in *Saccharomyces cerevisiae* metabolism as the temperature rises was apparent in data from Gilliland (7). The time required for cell replication was 42 hr at 80°C, 11 hr at 10°C, 3.2 hr at 18°C, and 1.6 hr at 28°C. Subsequent depletion of internal reserves resulted in cell death by loss of cell membrane integrity (1,3). As previously described, autofermentation can occur at temperatures above 10°C, which could augment the loss of viability (7).

When daily glycogen content of the yeast at the tested temperatures (Fig. 2) was examined, the resultant data did not form two distinct groups as for viability (Fig. 1). At any tested temperatures, the daily reduction in glycogen content was consistent throughout the 6 storage days. The daily rate of reduction increased as the temperature of storage increased (Fig. 2). At 1°C, yeast stored for 6 days decreased 5% in glycogen content, from 342 to 325 mg/L. Yeast stored at 25°C decreased 79%, from 342 to 75 mg/L. The final glycogen content after storage decreased approximately 15% for each 5°C rise in temperature.

Yeasts stored at 15, 20, and 25°C had final glycogen contents below 200 mg/L, the level suggested by Quain and Tubb (12) as the minimal yeast glycogen content required to permit normal fermentation upon pitching. Yeasts stored 6 days at 10°C also contained glycogen contents close to that value, making these yeasts a dubious choice for subsequent fermentation.

As previously determined (11), a direct correlation between glycogen content and yeast viability was found when the data from the storage temperature experiments were analyzed. The coefficient of correlation was identical (0.987) to that previously determined. However, the equation of the line of regression was different, the slope of the line was greater and the x-axis intercept was smaller. The equation of the line was $y = -36.7 + 4.2x$. The difference in the slope and intercepts of the regression analysis for the same species of yeast indicates that some other factor(s) are exerting a further influence on yeast viability and glycogen content, perhaps culture age, previous pitching level, glycogen level, or previous brew characteristics such as high gravity, time in fermenter, etc. Further research is continuing on this aspect.

The analysis of the yeast after storage for respiratory deficient (RD) mutants showed that at temperatures of storage higher than 15° C, the percentage of RD mutants in the population increased rapidly during the six days (Table I). At temperatures of storage of 1, 5, and 10° C, no measurable increase in RD mutants was recorded. It is an established fact that higher temperatures can increase the induction of RD mutants by increasing the growth rate and hence the rate of petite induction mutation (7). In addition, the ratio of RD mutants to normal yeast cells change due to the ability of the RD mutant to better survive the higher storage temperatures.

In examining the majority of the tested fermentation characteristics, it was found that the data collected for each yeast type (each storage temperature) could be placed into one of two groups. Fermentations pitched with yeast stored at 1, 5, and 10° C all exhibited similar responses for the tested characteristics, whereas fermentations pitched with yeast stored at 20 and 25° C exhibited another type of response. Yeast stored at 15° C fluctuated from one group to the other depending on the characteristic.

The levels of yeast in suspension (Fig. 3) during fermentations pitched with 1 and 5° C yeast were very similar to those normally recorded in nonstirred production fermentations. The numbers of yeast in suspension reached peak levels 48 to 50 hr after pitching, and that level was four times that of the original pitching rate. Between 55 to 118 hr after pitching the yeast began to rapidly flocculate out of suspension. Final levels of yeast in suspension were 10×10^6 cells/ml and 5×10^6 cells/ml for the 5° C yeast and 1° C yeast, respectively. Fermentations pitched with 10° C yeast exhibited a similar pattern and level of yeast in suspension during trial fermentations; however, the peak number of yeast in suspension was achieved 24 hr later than in fermentations conducted with 1 or 5° C. In addition, the rate at which the yeast began to flocculate out of suspension was slower. However, the final numbers of yeast in suspension were similar to final levels achieved in 1° C yeast fermentations.

Fermentations pitched with 15, 20, or 25° C yeast exhibited the second response type. During the first 20 hr of fermentation, the levels of yeast in suspension did not increase (Fig. 3). In fermentations pitched with 20 and 25° C yeast, the levels of yeast in suspension decreased by 45 to 55% from pitching levels. This phenomonen was directly related to the yeast cell viability after storage (Fig. 1). As previously described, viabilities were reduced 55, 63, and 68% for 15, 20, and 25° C yeast, respectively. The actual pitching rates with viable yeast were therefore 6.8, 5.5, and 4.8×10^6 yeast per milliliter. Almost immediately after pitching, the dead yeast cells dropped out of suspension, creating a thick layer of

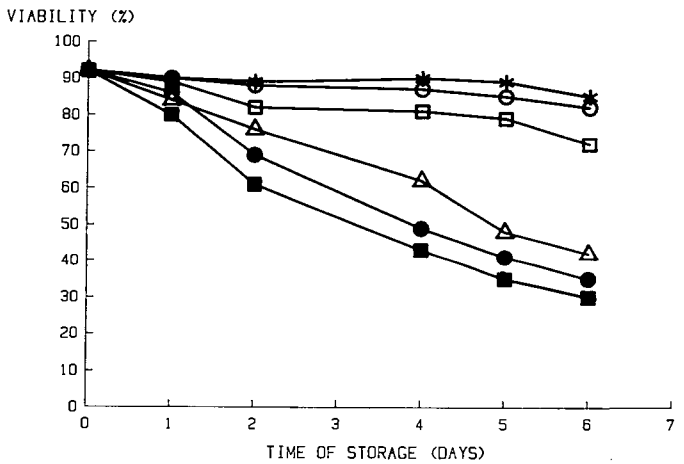

Fig. 1. Viability (%) of ale yeast stored at 1° C (*), 5° C (O), 10° C (□), 15° C (△), 20° C (●), 25° C (■).

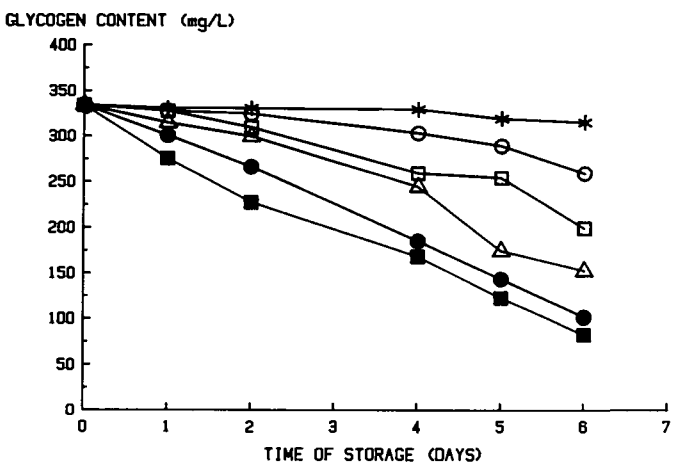

Fig. 2. Glycogen content (mg/L) of ale yeast stored at 1° C (*), 5° C (O), 10° C (□), 15° C (△), 20° C (●), 25° C (■).

TABLE I

Percent of Respiratory Deficient Mutants in Stored Yeast, and Final pH, Final Dimethyl Sulfide (DMS), and Final DMS Precursor (DMSP) Levels in Beer Produced by the Yeast after Storage

Temperature of Storage (°C)	Respiratory Deficient Mutants (%)	Final pH	Final DMS (μg/L)	Final DMSP (μg/L)
1	1.1	4.03	70	104
5	1.2	4.03	72	103
10	1.2	4.03	79	109
15	1.4	4.3	89	100
20	10	4.51	101	110
25	12	4.7	122	112

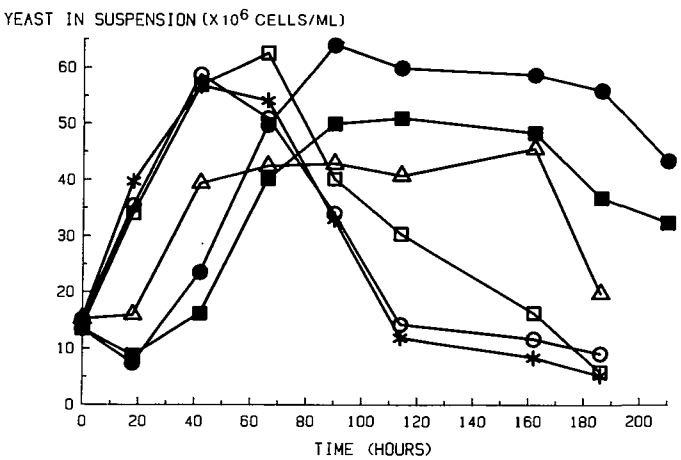

Fig. 3. Numbers of yeast in suspension (colony-forming units per milliliter) in trial fermentations pitched with ale yeast stored 6 days at 1° C (*), 5° C (O), 10° C (□), 15° C (△), 20° C (●), 25° C (■).

visible bottom sediment. In fermentations pitched with yeast stored at 20 or 25° C, the low viabilities were further complicated by very low levels of glycogen in the slurry (Fig. 2). The viable yeast required time to build up internal glycogen reserves for sterol synthesis before cell reproduction (12). As a result, the lag phase and subsequent logarithmic phase of growth were slower in fermentations pitched with 20 or 25° C yeast. In fermentations pitched with 15° C yeast, the rate of cell division, after a preliminary lag, was similar to that recorded in fermentations pitched with yeast stored at 1, 5, or 10° C. The peak numbers of yeast in suspension for fermentations pitched with yeast stored at 15, 20, or 25° C occurred between 90 and 100 hr after pitching, 50–60 hr later than in the other trial fermentations. In addition, the number of yeast in suspension remained high even after an additional 24 hr of cooling. This apparent loss of flocculence in the yeast has been previously noted in yeast weakened by exposure to abnormal pH, carbohydrate concentrations, etc. (5).

Attenuation and the number of yeast in suspension are intimately linked in fermentations (5). It was not surprising then that when the data were analyzed (Fig. 4), two groups containing fermentations exhibiting similar attenuation patterns were found. The members of each group were identical to the members of the groups recorded in terms of the yeast in suspension data (Fig. 3).

Fermentations pitched with yeast stored at 1, 5, and 10° C exhibited an attenuation pattern (Fig. 4) similar to that recorded in normal control fermentations. A rapid reduction in specific gravity began almost immediately after pitching and reached near-final attenuation 115 hr later. Fermentations with 5° C yeast exhibited a period of slow attenuation between 40 and 90 hr into fermentation. No explanation for this is available at this time. The final attenuations for 1, 5, and 10° C yeast fermentations were identical.

Fermentations pitched with yeast stored at 15, 20, and 25° C had progressively longer and more severe lag phases, slower rates of attenuation, and much higher final attenuation values than the fermentations pitched with 1, 5, or 10° C yeast. Final attenuations for 15, 20, and 25° C yeast fermentations were 91, 81, and 70%, respectively, if 1° C yeast fermentations were considered to attenuate 100%. Fermentations pitched with 20 and 25° C yeast were allowed to ferment 24 hr longer than the other trial fermentations, but no significant increase in attenuation occurred (Fig. 4). The final attenuation values in 15, 20, and 25° C yeast fermentations were higher than would be expected. Although these fermentations were initially slow to increase yeast cells in suspension, the yeast numbers eventually built up to levels comparable to those recorded in the control fermentations and fermentations pitched with 1, 5, or 10° C yeast. In addition, the numbers of yeast in suspension remained near the peak level for approximately 100 hr (Fig. 3). These two factors should have resulted in final attenuation values similar to those obtained in the other trial fermentations.

Analysis of the final beer revealed that the carbohydrate maltotriose was not utilized in the fermentations pitched with 15, 20, or 25° C yeast to the extent that it was in the other trial fermentations. This seems to indicate that a mutation was caused by storage at high temperature coupled with the nutrient-deficient conditions. Gilliland (7) pointed out that the second most troublesome mutation in ale yeast is involved with the genes necessary for maltotriose utilization. Many researchers point out a relationship between temperature and mutation (4); however, it was felt that the temperature of storage was not high enough to cause major genetic changes. Also it seems unlikely that the storage conditions, although severe in terms of yeast viability and glycogen content, could have restricted the production capabilities of the yeast cell to produce the enzyme required for maltotriose utilization. Subsequent investigation revealed no genetic changes. A more likely hypothesis is that the conditions could have caused structural changes in the yeast cell wall affecting the action of maltotriose permease. Nutrient-deficient conditions would have caused the yeast cell to cannibalize itself for any needed metabolites. Cell wall structure was affected in yeast stored at higher temperatures as evidenced by the loss in ability by these yeasts to flocculate. Flocculation is a function intimately associated with the yeast cell membrane structure (14). In addition, when yeast

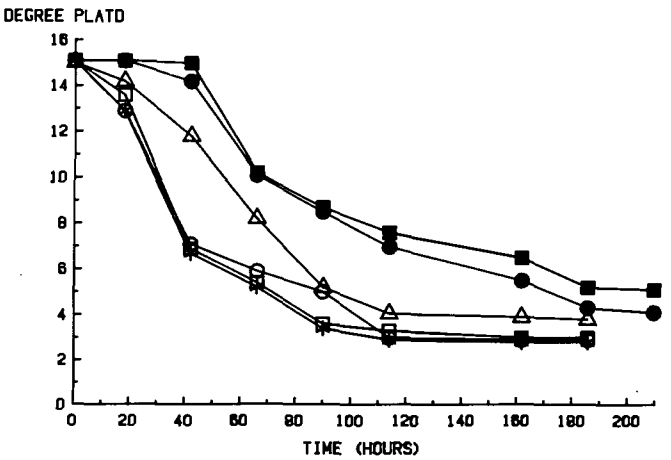

Fig. 4. Specific gravity (° P) of trial fermentations pitched with ale yeast stored 6 days at 1° C (*), 5° C (O), 10° C (□), 15° C (△), 20° C (●), 25° C (■).

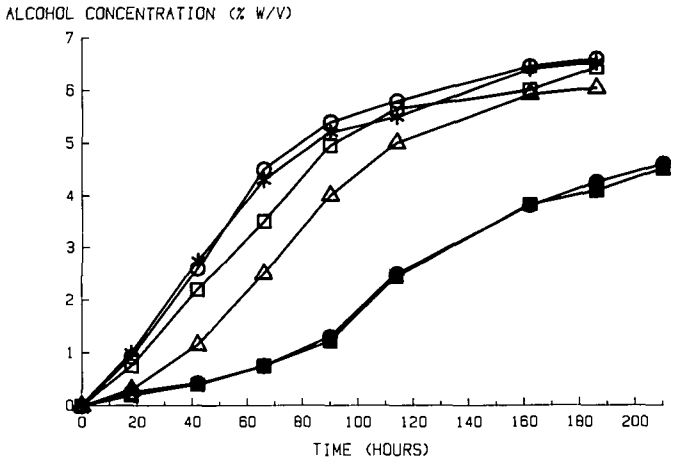

Fig. 5. Alcohol concentration (% w/v) in trial fermentations pitched with ale yeast stored 6 days at 1° C (*), 5° C (O), 10° C (□), 15° C (△), 20° C (●), 25° C (■).

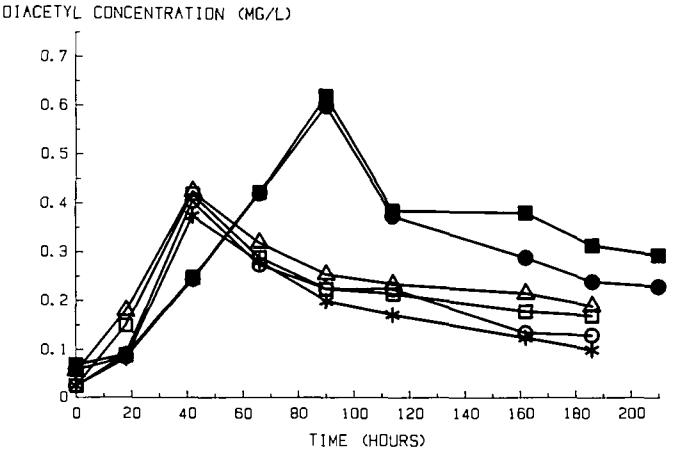

Fig. 6. Diacetyl concentration (mg/ml) in trial fermentations pitched with ale yeast stored 6 days at 1° C (*), 5° C (O), 10° C (□), 15° C (△), 20° C (●), 25° C (■).

collected from these fermentations was reused in another fermentation, the ability to fully utilize maltotriose and to flocculate at the end of fermentation returned.

When production of alcohol (Fig. 5) throughout the trial fermentations was plotted, again two response groups could be discerned. Fermentations pitched with 1, 5, 10, and 15°C yeast exhibited normal productions of alcohol. The final alcohol production in these four trial fermentations was similar.

Production of alcohol in 20 and 25°C yeast fermentations was slower than in the other trial fermentations and the final concentrations of alcohol were reduced 30%. This reduction was attributable to poor attenuation of these fermentations coupled with the requirement, due to severe underpitching, of more carbohydrates normally used in fermentation for the production of yeast cell biomass.

When production of the vicinal diketone diacetyl (Fig. 6) during the trial fermentations was examined, the resultant data grouped similarly to that for alcohol production. Production of diacetyl in fermentations with 1, 5, 10, and 15°C yeast followed the normal pattern recorded in production fermentations. Peak diacetyl production occurred about 40 hr after pitching, and that level was successively higher as the temperature of storage of the test yeast increased. In addition, reassimilation of diacetyl by the yeast during fermentation was less as the storage temperature of the yeast used to pitch the fermentations increased. This resulted in successively higher final diacetyl concentrations in the trial fermentations as the storage temperature increased. The difference in final diacetyl concentrations between 1 and 15°C yeast was 0.1 mg/L (1°C yeast was 0.09 mg/L and 15°C yeast was 0.19 mg/L).

Fermentations pitched with yeast stored at 20 or 25°C exhibited a diacetyl pattern similar in shape to that for the other trial fermentations. However, peak levels of diacetyl occurred 50 hr later, 90 hr after pitching, and that peak was twice that of the 1°C yeast fermentations. In addition, final diacetyl concentration was as much as three times that of 1°C yeast final concentrations.

When the final pH of the trial fermentations was examined (Table I), it was evident that no difference occurred for fermentations pitched with 1, 5, or 10°C yeast. Fermentations pitched with 15, 20, and 25°C yeast exhibited successively increasing final pH (4.31, 4.51, and 4.70, respectively).

The final concentration of dimethyl sulfide (DMS) recorded in the trial fermentations (Table I) differed little in the fermentations pitched with either 1, 5, or 10°C yeast. The final DMS concentration in fermentations pitched with 15°C yeast was 27% higher than in 1°C yeast fermentations, 20°C yeast fermentations 44% higher, and 25°C yeast fermentations 74% higher. These increases may have been due to the addition at pitching of large numbers of dead or damaged yeast from which intracellular DMS may have leaked. No significant differences were found in final DMSP concentration (Table I) in the trial fermentations.

CONCLUSIONS

Pitching yeast stored longer than 96 hr at temperatures above 5°C possess lower than acceptable glycogen contents and viabilities. Fermentations subsequently pitched with a yeast slurry stored at temperatures above 5°C have a longer lag phase, poor rate of attenuation and poor final attenuation, higher final DMS and diacetyl concentrations, higher final pH values, and lower final alcohol yields. As the temperature of storage is increased, these features become more pronounced.

ACKNOWLEDGMENTS

We thank Molson Breweries of Canada for their permission to publish these results and Margaret Morrison for her critical appraisal of the manuscript.

LITERATURE CITED

1. Andreason, A. A., and Stier, T. J. B. *J. Cell. Comp. Physiol.* 41:23, 1953.
2. Barton-Wright, E. C. *Wallerstein Lab. Commun.* 16:15, 1953.
3. Bloch, K., Borek, E., and Rittenberg, D. *J. Biol. Chem.* 162:441, 1946.
4. Brock, T. C. *Biology of Microorganisms.* Prentice Hall: Toronto, 1974.
5. Curtis, N. S., and Wenham, S. *J. Inst. Brew.* 64:421, 1958.
6. Farrell, J., and Rose, A. H. *Annu. Rev. Microbiol.* 21:101, 1967.
7. Gilliland, R. B. *Eur. Brew. Conv., Proc. Congr. 12th, Interlaken, 1969,* p. 303.
8. Jakobsen, M. *J. Inst. Brew.* (Austr. NZ Section), March, 1982, p. 132.
9. Kirsop, B. H. *J. Inst. Brew.* 80:252, 1974.
10. Masschelin, C. A., Dupont, J., Jeunehomme, C., and Devreux, A. *J. Inst. Brew.* 66:502, 1960.
11. McCaig, R., and Bendiak, D. S. *J. Am. Soc. Brew. Chem.* 43(2):114, 1985.
12. Quain, D. E., and Tubb, R. S. *Tech. Q. Master Brew. Assoc. Am.* 19(1):29, 1982.
13. Roessler, J. G. *Brew. Dig.,* December 1968, 43(1):57.
14. Stewart, G. G., Russell, I., and Garrison, I. F. *Am. Soc. Brew. Chem., Proc. 1973,* p. 100.