High-Gravity Brewing: Influence of Pitching Rate and Wort Gravity on Early Yeast Viability[1]

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ABSTRACT

Poor yeast crop viability has been reported in high-gravity brewing. When traditional pitching rates are used in high-gravity worts of 19.8–39° Plato, yeast viability is a problem within the first 24 hr of the fermentation. Both immediate and continued losses of viability and fermentative ability were observed; the duration and severity increased with increasing wort gravity. Surprisingly, viability losses were more severe when assayed by the methylene blue stain, traditionally a method that gives higher viabilities than enumerations based on colony-forming units. Under conditions of high-gravity brewing (high osmotic pressure), the methylene blue stain might indicate cell stress as well as viability. By either method, however, early losses in viability in high-gravity worts were significantly reduced by pitching at higher than usual pitching rates. The significance of this to high-gravity brewing is that such drastic cell death reduces the real pitching rate. Such lower pitching rates have been said to be more inefficient in the utilization of oxygen for lipid synthesis. As a result, sluggish starts, protracted fermentations, and oxygen-deficient yeast crops are more likely to occur.

Key words: *High-gravity brewing, Methylene blue, Pitching rate, Power, Yeast viability*

Traditionally, brewing worts of 11–12° Plato are fermented to produce beers that contain the required alcoholic concentration. Over the past few years, however, high-gravity brewing has become popular. This involves the production, fermentation, and storage of worts with substantially higher original gravities than the calculated original gravity based on the final beer.

Some product quality and economic advantages are: increased plant efficiency; reduced energy, labor and capital costs; use of higher adjunct ratios; improved beer smoothness; improved flavor and haze stability; and increased ethanol yields per unit of fermentable extract (3,10,12,24–26,29–32).

Advantages are realized only when four conditions are met. First, the time required for fermentation must not be markedly increased. Second, the extent of fermentation must be satisfactory. Third, the flavor of the beer must remain acceptable. And fourth, the yeast crop must be maintained at high viability. For these reasons, the current commercial limit in high-gravity brewing is from 16° P (3,12,28,31) to 18° P (10,12,33). Above this range, beer flavor is altered by dramatic increases in ester production, (especially isoamyl acetate and ethyl acetate) (2). Yeast viability and fermentation problems also arise. Pfisterer and Stewart (24), for example, reported incomplete attenuation of 16° P worts by several top-cropping ale strains and of 18° P worts by bottom-cropping strains. Day et al (7) and White (29) found that, although beers having up to 10% ethanol (v/v) could be produced, the resulting yeast crop was of such poor viability that its reuse was precluded. Presumably, this was due to problems of ethanol toxicity.

In the present study, the influences of wort original gravity and pitching rate on yeast viability and fermentative ability were examined. Instead of focusing on the state of the yeast at the end of high-gravity fermentations, however, their performance within the first crucial 24 hr of fermentation was examined. Concrete evidence supports the need for higher pitching rates to prevent early losses in viability and fermentative power in high-gravity worts. The significance of this observation to high-gravity brewing is discussed.

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EXPERIMENTAL

Microorganism

A production strain of *Saccharomyces uvarum* (carlsbergensis) was used. Fresh slurries of this commercial lager yeast were collected just before use.

High-Gravity Worts

An 11.5° P corn-grit adjunct commercial wort was used. All wort was autoclaved so that protein could be removed. This allowed yeast cell mass assessment without trub interference. Fermentations have been shown to be normal (*unpublished*). For preparing worts of higher original gravity, Casco syrup no. 1636U was added (Canada Starch Company, Limited). The manufacturer's carbohydrate specifications for this syrup are 19.4% DP4+, 10.7% DP3, 39.0% DP2, and 30.9% DP1.

Fermentation Conditions

One- and two-liter Wheaton fermentors were used. Fermentations were stirred at 90 rpm by the use of a Wheaton model III Biostir 6. Temperature was maintained at 14°C by a Haake model G circulator, with water as the coolant. Anaerobic conditions were assured by continual flushing of the headspace with nitrogen gas (oxygen free-Weld-Arc, Saskatoon, Saskatchewan).

Viable Counts

The membrane-filtration technique for viable counting was used as previously described (14). Triplicate aliquots of appropriate dilutions were filtered through sterile 0.45-μm Gelman 47-mm membranes. Sterile 0.1% peptone water was used to prepare all dilutions as well as to rinse holders. Membranes were then incubated for three days at 27° C on plates of YEPD agar (1% yeast extract, 2% peptone, 2% dextrose, and 2% agar at pH 7.0). Percent viability relates a particular plate count during the experiment to the viable cell number at time zero.

Methylene Blue Counts

The methylene blue counting technique used was based on the EBC Analytica method (8). Sodium citrate dihydrate (2 g) was added to 10 ml of a 0.1% aqueous methylene blue solution and made up to 100 ml with distilled water. An equal volume of this solution was then added to an equal volume of an aqueous suspension of the yeast to be counted. For each determination, at least 800 yeast cells were counted in an improved Neubauer counting chamber (American Optical Corporation). Cells that stained blue were counted as dead, and unstained cells were considered to be viable. Percent viability was then calculated.

Total Solids and Dry-Weight Determinations

Wort samples were centrifuged for 15 min at 4°C in a Sorvall RC2-B centrifuge at a relative centrifugal force of 12,100 × g. From the resulting supernatant fluid, duplicate 4-ml samples were dried in preweighed aluminum foil pans to constant weight at 105° C, and the total wort solids were calculated in grams per 100 ml.

For dry-weight determinations, the resulting cell pellet was washed three times in $M/15$ KH_2PO_4 (pH 4.5) and then resuspended in the same buffer. Duplicate 3-ml samples of the resuspensions and of the resuspending KH_2PO_4 were transferred to preweighed aluminum foil pans. Pans were dried to constant weight at 105° C, corrected for buffer weight, and cell dry weight (mg/ml) was calculated.

Manometric Analysis

The fermentative power of yeast samples (washed three times in *M*/15 pH 4.5 KH_2PO_4 buffer), was determined with the Warburg respirometer using EBC Analytica Microbiologica Manometric techniques (9). The single alteration to the published method was the use of 30° C temperature instead of 28° C. Fermentative power, written as $Q^{N_2}_{CO_2}$ was expressed as the number of microliters of CO_2 given off under a nitrogen atmosphere per hour per milligram of dry weight of yeast.

RESULTS AND DISCUSSION

During preliminary investigations into the fermentation of high-gravity worts, losses in yeast viability frequently occurred between the time of pitching and the 24-hr mark (*unpublished*). The extent of these losses, however, seemed to decrease at higher pitching rates. Such observations have not been previously reported.

Table I summarizes yeast viability during the first 12 hr of fermentation for experiments done over three one-week periods. Pitching rates of 6.0×10^6 colony-forming units (CFU) per milliliter, 14.9×10^6 CFU per milliliter, and 40.0×10^6 CFU per milliliter were used over a wide range of wort original gravities. In terms of cell viability, the two lower pitching rates and the highest pitching rate are clearly different. At 40×10^6 CFU per milliliter, no cell death occurred in worts up to 31.4° P original gravity only at 38.8° P did extensive cell death occur at either time period. At the two lower pitching rates, the results were markedly different. At 0.5 hr, viabilities ranged from 96 to 45% for both the 6.0×10^6 and 14.9×10^6 CFU per milliliter pitching rates. By the 12-hr mark, although cell numbers had predictably increased at the lower gravities, there was an even further decrease in viability at higher gravities, with lows of 20.0 and 10.1% viability being reached at the 6.0×10^6 and 14.9×10^6 CFU per milliliter pitching rates, respectively.

The methylene blue stain was also used in these experiments as an alternative indicator of early cell viability. Traditionally, this method gives consistently higher viable cell counts than plating techniques (8,11,13,19,20,23). The difference is attributed to the large percentage of cells that remain unstained by methylene blue yet are unable to form microcolonies when isolated into sterile wort droplets (21) or when incubated on a slide culture (8). As Table II, illustrates, viability as assayed by this method was also severely affected as wort original gravity increased, especially at the lower pitching rates. In each case, however, the extent of cell death was much greater when measured by the methylene blue stain as

TABLE I
Influence of Pitching Rate and Wort Original Gravity on Yeast Viability 0.5 and 12.0 Hr After Pitching

Pitching Rate (CFU/ml)[a]	Wort Original Gravity (°P)	Yeast Viability After 0.5 Hr (%)	Yeast Viability After 12 Hr (%)[b,c]
6.0×10^6	11.9	93.3	161.7
	19.8	81.7	136.7
	24.8	76.7	75.0
	31.0	58.3	38.3
	39.0	45.0	20.0
14.9×10^6	11.5	96.0	147.0
	19.1	100.0	88.6
	23.9	87.2	47.0
	30.2	69.8	14.8
	38.6	57.0	10.1
40.0×10^6	12.1	117.8	151.8
	19.8	118.0	149.3
	24.0	112.8	120.8
	31.4	111.3	105.0
	38.8	63.3	75.8

[a] CFU = colony-forming units.
[b] Thirteen hours for the 40.0×10^6 CFU/ml pitching rate.
[c] Percent viability relates a particular plate count during the experiment to the viable cell number at time zero.

TABLE II
Cell Viability by Methylene Blue Staining

		Methylene Blue Stain (0.5 hr)		
Pitching Rate (CFU/ml)	Wort 0.6[a] (°P)	Total Cells per Milliliter ($\times 10^{-6}$)	Unstained (Viable) Cells per Milliliter ($\times 10^{-6}$)	Variability (%)
6.0×10^6	11.9	10.8	9.7	89.8
	19.8	10.0	7.9	79.0
	24.8	10.7	7.4	69.2
	31.0	11.1	4.0	36.0
	39.0	10.3	1.3	12.6
14.9×10^6	11.5	20.6	19.3	93.6
	19.1	20.8	15.4	74.0
	23.9	21.7	10.9	50.2
	30.2	19.2	5.6	29.2
	38.6	19.5	1.5	7.7
40.0×10^6	12.1	77.0	70.8	91.9
	19.8	80.1	70.0	87.4
	24.0	78.8	65.3	82.9
	31.4	79.3	59.5	75.0
	38.8	77.9	50.9	65.3

[a] Original gravity.

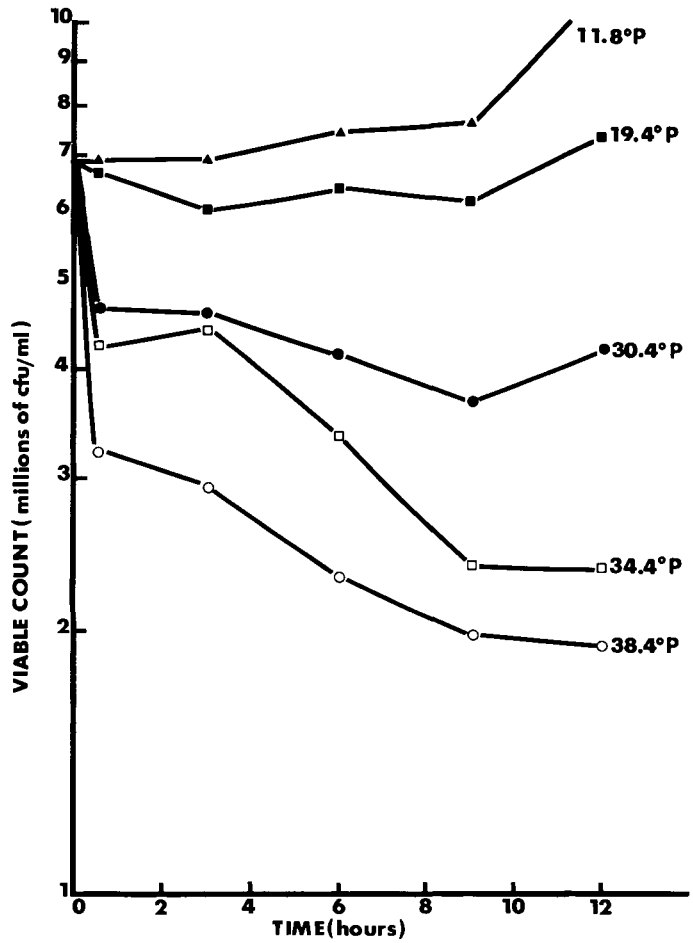

Fig. 1. Yeast viability (millions of colony-forming units per millilter) over the first 12 hr in 11.8–38.4° P worts pitched at 6.8×10^6 colony-forming units per milliliter.

opposed to the percent reduction in the colony-forming units (Table I vs Table II). This is the opposite of what might have been expected. In fact, the actual viable cell counts, when determined by membrane filtration, were considerably higher at several of the higher wort gravities for the lower pitching rates than they were when estimated by the methylene blue stain. This indicates that many of the stained cells were still capable of forming colonies and, as such, the methylene blue stain under such conditions may serve as an indicator of cell stress as well as viability.

Clearly, significant losses in yeast viability occur in very high-gravity worts, losses that can be partly offset by the use of higher pitching rates. An interesting question was, then, whether or not yeast cells unable to form colonies on a plate were still able to contribute to fermentation. Were they also metabolically dead?[2] To answer this, worts ranging from 11.8 to 38.4° P were pitched at 6.8×10^6 and at 63.2×10^6 CFU per milliliter. In addition to following yeast viability by membrane filtration, the fermentative power ($Q^{N_2}_{CO_2}$) of the pitched yeast was determined at the 0.5- and 24.0-hr points of fermentation.

As anticipated, the losses in viability in yeasts used to determine fermentative power were more severe at the lower pitching rate. At 0.5 hr, for example, the percent viability varied from 107 to 47% at the 6.8×10^6 CFU per milliliter pitching rate (Fig. 1), but only from 95 to 83% at the 63.2×10^6 CFU per milliliter pitching rate (Fig. 2). Methylene blue stains of 0.5-hr samples yielded results similar to the pattern discussed earlier (*data not shown*).

Figure 3 illustrates CO_2 production by washed yeast from the two different pitching rates 0.5 hr after pitching. Clearly, the decrease in the rate of CO_2 production as the original gravity of the wort increases is less severe at the higher pitching rate. $Q^{N_2}_{CO_2}$ values, relative to that of the pitching yeast, ranged from 86 to 8% at the 6.8×10^6 CFU per milliliter pitching rate compared with 103 to 45% at the 63.2×10^6 CFU per milliliter pitching rate (Table III). The superior fermentative ability of the yeast at the higher pitching rate became even more apparent by the 24-hr mark (Fig. 4). The $Q^{N_2}_{CO_2}$ values here ranged from 103 to 3% at the lower pitching rate, compared with a range of 121 to 89% at the higher pitching rate. All but the $Q^{N_2}_{CO_2}$ value of the yeast in the 38.4° P wort was below that

[2] Defined as unable to ferment glucose to CO_2 (and ethanol).

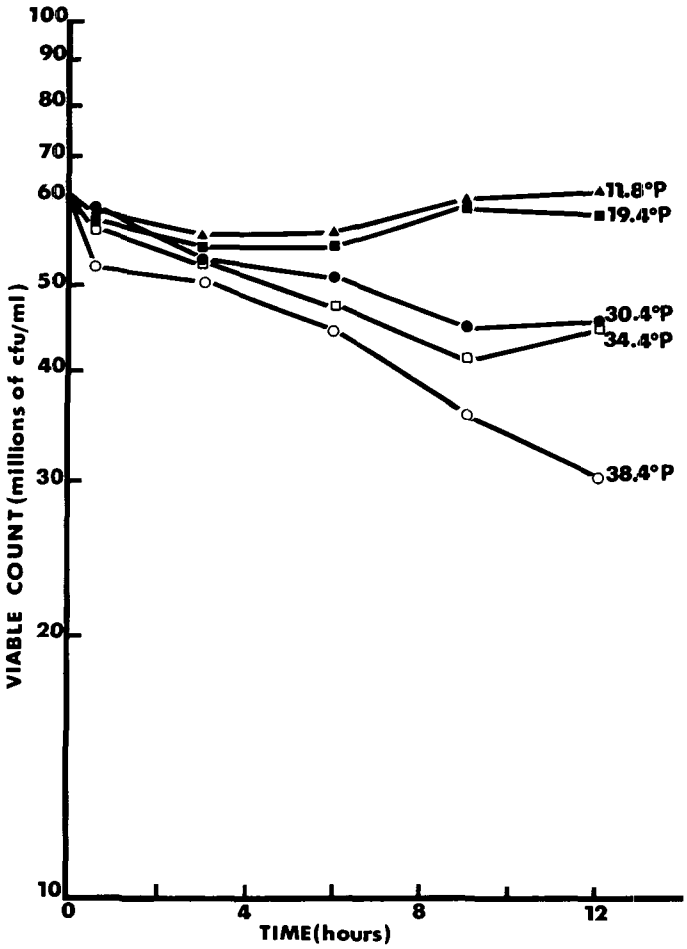

Fig. 2. Yeast viability (millions of colony-forming units) over the first 12 hr in 11.8–38.4° P worts pitched at 63.2×10^6 colony-forming units per milliliter.

TABLE III
$Q^{N_2}_{CO_2}$ Values of Yeast at 0.5 Hr in 11.8–38.4°P Worts Pitched at 6.8×10^6 and $63.2 \times$ CFU[a]/ml

Wort Gravity (°P)	$Q^{N_2}_{CO_2}$[b] 6.8×10^6 CFU/ml[c]	$Q^{N_2}_{CO_2}$[b] 63.2×10^6 CFU/ml[d]	Percent $Q^{N_2}_{CO_2}$ Relative To Pitching Yeast 6.8×10^6 CFU/ml	Percent $Q^{N_2}_{CO_2}$ Relative To Pitching Yeast 63.2×10^6 CFU/ml
11.8	190	194	86	97
19.4	106	206	48	103
30.4	84	135	38	68
34.4	66	125	30	63
38.4	18	90	8	45

[a] CFU = colony-forming units.
[b] Microliters of CO_2 per hour per milligram.
[c] $Q^{N_2}_{CO_2}$ of pitching yeast = 221 μl CO_2 per hour per milligram.
[d] $Q^{N_2}_{CO_2}$ of pitching yeast = 200 μl CO_2 per hour per milligram.

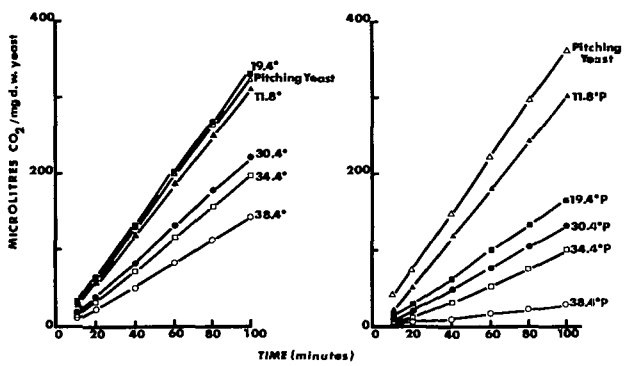

Fig. 3. Rates at 0.5 hr of CO_2 production (μl/mg CO_2) of washed yeast from 11.8–38.4° P worts.

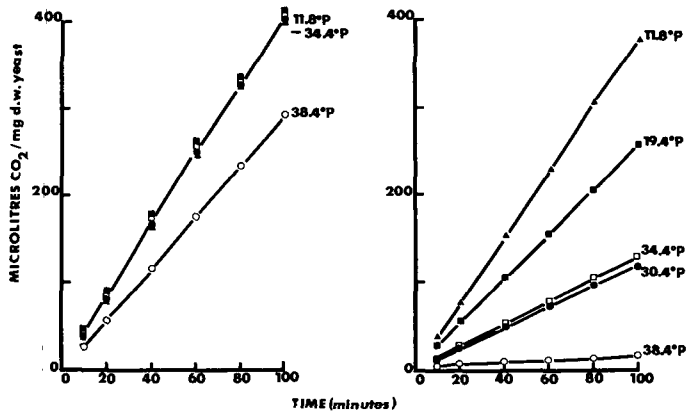

Fig. 4. Rates at 24.0 hr of CO_2 production (μl/mg CO_2) of washed yeast from 11.8–38.4° P worts.

found in the original pitching yeast (Table IV). Obviously, metabolic death also occurs at higher wort gravities, but to a much reduced extent at the higher pitching rate.

When the 0.5-hr $Q^{N_2}_{CO_2}$ values are compared to the values of the pitching yeast by linear regression, the improved fermentative properties of yeast at the higher pitching rate are clearly seen (Fig. 5). At a particular wort gravity, the fermentative vigor of the yeast pitched at the higher rate, on a per-milligram basis, is significantly superior to that of the yeast at the lower pitching rate. In addition, the steeper negative slope of the plot for the lower pitching rate causes the extent of the difference between the two pitching rates to become even greater as wort gravity increases. This was reflected by the fact that none of the wort solids had been fermented at the lower pitching rate in 24 hr in the 30.4, 34.4, or 38.4° P worts. In addition, attenuation was always greater at the higher pitching rate (Table V). Attempts by adaptation to overcome these early losses in viability at the lower pitching rate in 34.4° P wort through five successive transfers of the yeast proved unsuccessful. Although the extent of death did not appear to decrease at the 0.5-hr point by repitching, the eventual degree of cell death by the 9-hr mark was significant in all cases (averaging 51% over the five runs).

The relevance of these observations to high-gravity brewing became apparent on examination of the importance of pitching rate in brewing. Since the first publications on high-gravity brewing appeared, it has been recommended that the pitching rate be increased in proportion to the increase in gravity (26,28,30,32). The mechanism underlying the importance of pitching rates in brewing, has become clear only in recent years, following studies on the relationship between yeast growth, sugar utilization, and ethanol formation. The traditional belief that yeast growth and wort attenuation are separate and successive (27) is now known to be incorrect. Instead, results indicate that wort-sugar utilization and biomass production are definitely coupled with the specific rate of sugar utilization by growing cells substantially higher than that of nongrowing cells (15,17,27).

This close relationship between cell-mass increase and sugar utilization dictates that, if any wort fermentation is to be rapid, growth must continue essentially throughout the period of sugar utilization. Therefore, because of the additional extract in high-gravity brewing, yeast growth must continue for a sufficient period of time. To ensure rapid attenuation, the time required must be in excess of that required in normal-gravity brewing.

The wort component most often involved in limiting yeast growth is oxygen, the importance of which was recently reviewed by Kirsop (18). Oxygen is required by brewing yeast for the synthesis of sterols and unsaturated fatty acids (1). In brewery fermentations, yeast growth and hence the more rapid rate of attenuation, usually decrease after a minimal concentration of these lipids is reached (4–6). At lower pitching rates, yeast uses wort-dissolved oxygen less efficiently, the amount of cell-mass synthesis possible is decreased and the rate of the fermentation is slowed (13,16). Kirsop (16) has shown with worts of only 15° P that, when the pitching rate was reduced from 5 g/L to 1.5 g/L, both the rate and extent of wort attenuation decreased considerably as a result of the decreased amount of yeast growth arising from the less efficient use of oxygen for lipid synthesis.

The results of our research suggest that the use of traditional pitching rates in the fermentation of very high gravity worts will result in the early death of a significant portion of the yeast population. This results in a much reduced effective or actual pitching rate (up to 10-fold in the results presented here), and in yeast that will be less able to utilize oxygen in wort for the production of lipids. This will therefore limit the potential for cell mass synthesis and thereby lower the rate (and extent) of fermentation. Using a pitching rate much higher than that traditionally used, the degree of early losses in viability is significantly reduced, thereby increasing the ability of the yeast to utilize wort oxygen.

Obviously, an increase in pitching rate alone will not result in the full attenuation of high-gravity worts in the order of the ones used

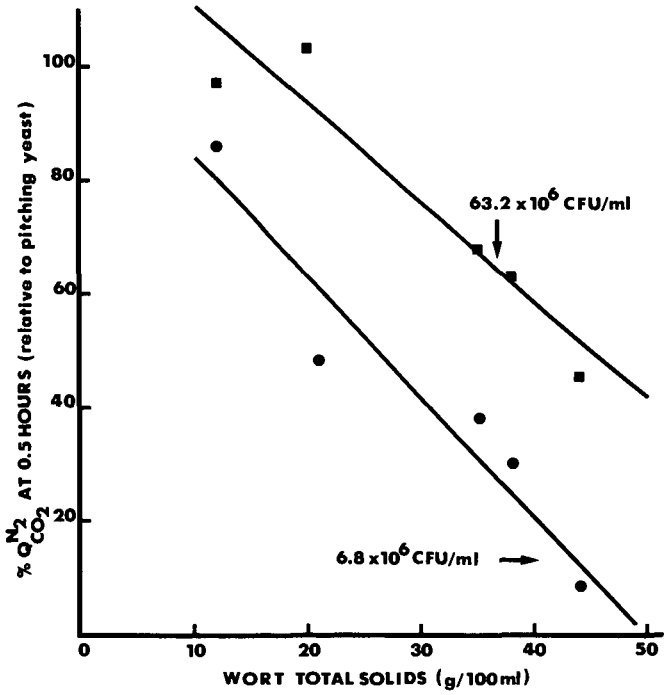

Fig. 5. Percent $Q^{N_2}_{CO_2}$ at 0.5 hr (relative to pitching yeast) of yeast pitched at 6.8×10^6 and 63.2×10^6 colony-forming units per milliliter vs starting wort original gravity.

TABLE IV
$Q^{N_2}_{CO_2}$ Values (24 hr) of Yeast in 11.8–38.4° P Worts Pitched at 6.8×10^6 and 63.2×10^6 CFU[a]/ml

Wort Gravity (°P)	$Q^{N_2}_{CO_2}$[b] 6.8×10^6 CFU/ml[c]	$Q^{N_2}_{CO_2}$[b] 63.2×10^6 CFU/ml[d]	Percent $Q^{N_2}_{CO_2}$ Relative To Pitching Yeast 6.8×10^6 CFU/ml	Percent $Q^{N_2}_{CO_2}$ Relative To Pitching Yeast 63.2×10^6 CFU/ml
11.8	227	237	103	119
19.4	152	238	69	119
30.4	70	236	32	118
34.4	79	241	36	121
38.4	7	177	3	89

[a] Colony-forming unit.
[b] Microliters of CO_2 per hour per milligram.
[c] $Q^{N_2}_{CO_2}$ of pitching yeast = 221 μl CO_2 per hour per milligram.
[d] $Q^{N_2}_{CO_2}$ of pitching yeast = 200 μl CO_2 per hour per milligram.

TABLE V
Attenuation of 11.8–38.4°P Worts Pitched at 6.8×10^6 and 63.2×10^6 CFU[a]/ml

Starting Wort Gravity (°P)	Starting Total Solids (g/100 ml)	Total Solids After 24 Hr 6.8×10^6 CFU/ml	Total Solids After 24 Hr 63.2×10^6 CFU/ml	End Total Solids 6.8×10^6 CFU/ml	End Total Solids $63.2 \times 10_6$ CFU/ml
11.8	12.2	10.3	4.8	4.2	4.1
19.4	20.9	19.3	12.0	6.7	6.4
30.4	35.1	35.0	24.9	24.2	22.7
34.4	37.9	37.7	29.5	27.2	25.6
38.4	43.7	43.8	35.9	30.9	28.4

[a] Colony-forming unit.

in this paper. Other necessary modifications will be the subject of a future report.

At this time, neither the causes of the early losses in viability nor the mechanism of the protective effect of higher pitching rates is clear. It does seem certain that the losses in viability are not the result of accumulation of intracellular ethanol resulting from increased osmotic pressure (22), due to the immediate nature of much of the death, and because no drop in the wort total solids occurred at all within the first 24 hr in the cases with the most severe amount of death. The involvement of osmotic pressure, per se, cannot yet be ruled out, but the possibility of death resulting specifically from severe shock excretion alone seems unlikely as the osmotic pressure of these high-gravity worts (21) actually comes closer to the internal osmotic pressure of yeast harvested from 12° P beer. Additional studies are required to further explore these phenomena, and to determine whether these observations pertain to all commercial brewers yeasts.

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LITERATURE CITED

1. Anderson, A. A., and Stier, J. B. *J. Cell. Comp. Physiol.* 41:23, 1953.
2. Anderson, R. G., and Kirsop, B. H. *J. Inst. Brew.* 80:48, 1974.
3. Anonymous. *Brew. Distill. Int.* 9:22, 1979.
4. Aries, V., and Kirsop, B. H. *J. Inst. Brew.* 83:220, 1977.
5. Aries, V., and Kirsop, B. H. *Eur. Brew. Conv., Proc. Congr. 16th, Amsterdam, 1977*, p. 255.
6. Aries, V., and Kirsop, B. H. *J. Inst. Brew.* 84:118, 1978.
7. Day, A., Anderson, E., and Martin, P. A. *Eur. Brew. Conv., Proc. Congr. 15th, Nice, 1975*, p. 377.
8. EBC: Yeast Group. *J. Inst. Brew.* 68:14, 1962.
9. EBC Analytica Microbiologica. *J. Inst. Brew.* 87:303, 1981.
10. Gemin, C. *Tech. Q. Master Brew. Assoc. Am.* 11:21, 1974.
11. Gilliland, R. B. *J. Inst. Brew.* 65:424, 1959.
12. Hackstaff, B. W. *Tech. Q. Master Brew. Assoc. Am.* 15:1, 1978.
13. Hall, J. F. *J. Inst. Brew.* 60:482, 1954.
14. Ingledew, W. M., Burton, J. D., Hysert, D. W., and Van Gheluwe, G. J. *Am. Soc. Brew. Chem.* 38:125, 1980.
15. Kirsop, B. H. *J. Inst. Brew.* 80:225, 1974.
16. Kirsop, B. H. *Brew. Dig.* 53(7):28, 1978.
17. Kirsop, B. H. *Eur. Brew. Conv. Fermentation Storage Symp. 1978*, p. 3.
18. Kirsop, B. H. *Topics Enz. Ferm. Biotech.* 6:79, 1982.
19. Mills, D. *Food Res.* 6:361, 1941.
20. Oppenworth, W. F. *Brauwissenschaft* 11:113, 1958.
21. Owades, J. *Tech. Q. Master Brew. Assoc. Am.* 18:163, 1981.
22. Panchal, C. J., and Stewart, G. G. *J. Inst. Brew.* 86:207, 1980.
23. Parkkinen, E., Oura, E., and Suomalainen, H. *J. Inst. Brew.* 82:283, 1976.
24. Pfisterer, E., and Stewart, G. G. *Eur. Brew. Conv., Proc. Congr. 15th, Nice, 1975*, p. 255.
25. Pfisterer, E., and Stewart, G. G. *Brew. Dig.* 51(6):34, 1976.
26. Schaus, O. O. *Tech. Q. Master Brew. Assoc. Am.* 8:7, 1971.
27. Searle, B. A., and Kirsop, B. H. *J. Inst. Brew.* 85:342, 1979.
28. Skinner, K. *The Brewer* 63:6, 1977.
29. White, F. H. *Inst. Brew.* (Aust. NZ sect.). *Proc. 15th Conv., Christchurch, NZ, 1978*, p. 133.
30. Whitear, A. *The Brewer* 63:60 (1977).
31. Whitworth, C. *Eur. Brew. Conv., Fermentation Storage Symp., 1978*, p. 155.
32. Whitworth, C. *The Brewer* 66:339, 1980.
33. Witt, P. R., and Blythe , P. *J. Am. Soc. Brew. Chem.* 34:76, 1976.

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