

Changes in Brewer's Yeast During Storage and the Effect of These Changes on Subsequent Fermentation Performance¹

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ABSTRACT

A slurry of brewer's yeast in beer was stored in two, 200-bbl cylindroconical vessels at 1.6°C. One was maintained full, while 34% of the yeast was removed from the other vessel after 24 hr. The yeast was stored in both vessels for a total of 80 hr. Samples were removed at intervals and analyzed for glycogen, trehalose, and viability. Aliquots of each yeast sample were pitched into 2 L of wort. Yeast glycogen and trehalose declined only slightly in the full vessel, but in the other vessel, glycogen declined significantly after removal of a portion of the yeast. Viability of the yeast stored by either method remained between 90 and 96%. Fermentations of the yeast stored by either method showed no relationship between yeast fermentation performance and glycogen or trehalose content. The fermentation performance of yeast stored under these industrial conditions was independent of glycogen or trehalose content and several reasons for this are discussed.

Key words: Brewer's yeast, Fermentation, Glycogen, *Saccharomyces cerevisiae* var. *uvarum*, Storage, Trehalose

Glycogen is the major carbohydrate reserve of the yeast cell. This polysaccharide consists of glucose units linked by α -1,4 bonds, which are branched by α -1,6 bonds (10). Glycogen is generally regarded as a source of both metabolic energy and carbon skeletons that can be mobilized rapidly for biochemical reactions. Quain and Tubb (17) divided glycogen metabolism during primary beer fermentation into three phases. During the first phase, when the yeast is pitched into air-saturated wort, there is a rapid decline of yeast glycogen. During the second phase, glycogen is synthesized and stored both during and after the cessation of yeast growth. In this second phase, glycogen can account for up to 50% of the yeast dry weight (7,12,17). In the final phase of metabolism, glycogen declines in the yeast cell as carbon becomes limiting (7,16,19).

The rapid decline of glycogen in the first stage of fermentation coincides with the utilization of oxygen and the de novo synthesis of lipids. Quain and Tubb (17) concluded from their studies on the fermentation performance of yeast containing high and low glycogen levels, that glycogen is utilized as an energy source during the first part of fermentation to synthesize the lipids essential for growth. Furthermore, these workers suggested that the glycogen level of the pitching yeast would affect the subsequent fermentation and could be used as an indicator of yeast performance (17,18). They recommended that yeast pitching rates should be adjusted in accordance with yeast glycogen content. Following this observation, several others found a similar correlation between yeast glycogen and fermentation performance (11,13). However, these studies on the effect of glycogen on yeast performance were done with yeast grown or maintained under laboratory conditions. In this study, the impact of industrial yeast storage conditions on the level of both intracellular glycogen and trehalose was assessed, as well as the relationship between these levels of carbohydrates and subsequent fermentation performance.

EXPERIMENTAL

Brewer's Yeast

A production strain of *Saccharomyces cerevisiae* var. *uvarum* was used.

Yeast Storage

A slurry of production yeast in beer (approximately 18% solids) was stored in two, 200-barrel stainless steel cylindroconical vessels maintained at 1.6°C. One remained full for 80 hr, while the other had 34% of the slurry removed after 24 hr and was maintained 66% full for the remaining 56 hr. The vessel had a headspace of 18 in. when full and 40 in. when 66% full. The vessels were stirred continuously at 400 rpm by a 16-in. single-blade impellor. The lid of the vessel was closed during storage, but excess carbon dioxide was able to escape through a vent. During storage, samples were taken at intervals and analyzed for glycogen, trehalose, and viability. A further portion of yeast was used to pitch 2 L "mini-fermentations" of production wort.

Because this work was done using production equipment, each experiment was conducted separately. The data reported here are from two pairs of experiments that showed essentially the same results.

Analyses

Duplicate aliquots of yeast cells (10 mg dry wt) were washed twice in 0°C water and glycogen determined by the enzymic method of Becker (4) as modified by Bergmeyer and Bernt (5). Trehalose was determined by the method of Trevelyan and Harrison (21). Yeast viability was determined using a slide culture technique (1).

Mini-Fermentations

To avoid any variation in wort composition during the course of these experiments, sufficient 15° Balling production wort (70% malt, 30% rice) was bottled, pasteurized, and stored at 4°C. Yeast samples were pitched at 20×10^6 /ml into 2 L of aerated wort containing 8 ppm oxygen. The wort was allowed to settle for 16 hr at 14°C before pitching to control the level of trub. The fermentor, which consisted of a 2-L Pyrex cylinder (Corning Glass Works, Corning, NY) sealed with a fermentation airlock, was maintained at 14°C for seven days in a low-temperature incubator (model 815; Precision Scientific group, Chicago, IL). Yeast cells were counted twice a day during the first three days of fermentation and once a day thereafter using a particle counter (model 112; Particle Data Inc., Elmhurst, IL). Specific gravity was measured daily using a density meter (model DMA 55; Mettler-Paar, Hightstown, NJ) and then converted to degrees Balling using tables (2).

Graphs were constructed of degrees Balling versus time and yeast cell count versus time. Hours to reach 6° Balling is 4° Balling above attenuation. In production, fermentations are cooled when they reach 6° Balling. Consequently, this measurement makes laboratory data translatable into production results. Peak cell count, which represents the maximum number of yeast cells in suspension, was read from the highest reading on the growth curve.

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RESULTS AND DISCUSSION

Previous studies have shown a significant decline of yeast glycogen when yeast slurries were stirred during storage (11,12). This observation prompted these workers to recommend that yeast be stored in a static condition, under beer, to preserve its glycogen content. In the present study, the slurry in the full vessel was stirred during storage, yet the glycogen content declined only slightly (Fig. 1). It is likely that the decline in glycogen reported in previous studies was caused by incorporation of oxygen into the yeast slurry by the laboratory equipment used for storage. The relatively inefficient agitation of our vessel would have entrained minimal oxygen. In the partially full vessel, which was also stirred, yeast glycogen declined from 15.6 to 9.0% dry weight after removal of 34% of the yeast at 24 hr (Fig. 1). This decline in glycogen can be attributed to exposure of the yeast to oxygen when air was drawn into the vessel during yeast removal. Also, agitation with the smaller volume was more efficient, and would have incorporated air into the slurry. Oxygen has been shown to be detrimental to many microorganisms (13), and exposure of stored yeast to oxygen

causes a loss of viability and a decrease in glycogen content (11,12,17).

Trehalose is a disaccharide that, like glycogen, is accumulated during periods of slow growth or starvation (20). Pringle and Lillie (15) showed that during prolonged starvation, the survival of yeast ultimately depended on the trehalose level and so this could also be important during brewer's yeast storage. In our study, there was little overall change in yeast trehalose during storage in the full vessel, but in the partially full vessel it declined from 5 to 3% dry weight (Fig. 2). Quain, Thurston, and Tubb (19) also showed that trehalose levels changed very little during fermentation. Therefore, there does not appear to be any relationship between trehalose content and yeast fermentation performance.

Previous studies have shown that faster fermentations were obtained with yeast containing higher levels of glycogen (11,12,17). Quain, Thurston, and Tubb (19) observed that exposure of yeast to aerated wort caused a rapid breakdown of glycogen as lipids were synthesized; yet, there was no apparent utilization of exogenous fermentable carbohydrate from the wort. They concluded that glycogen supplied the energy and carbon for lipid synthesis and so explained how glycogen was related to fermentation performance.

The initial differences in the fermentation times between the two

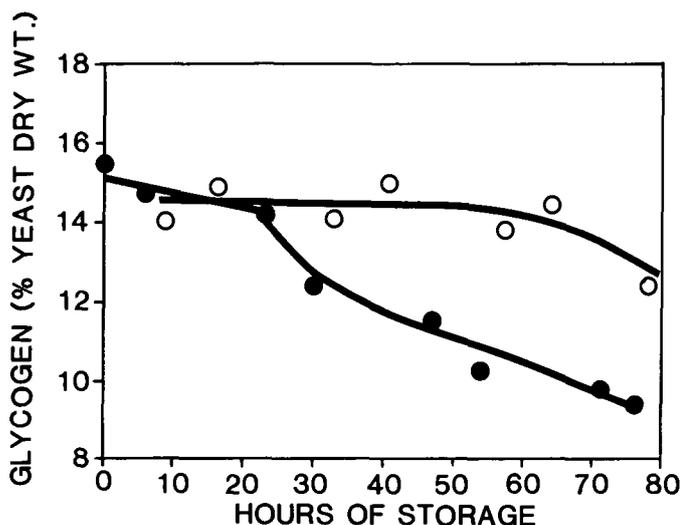


Fig. 1. Glycogen content of yeast during storage in a 200-bbl vessel. One vessel remained filled with yeast slurry over an 80-hr period (o), while the other vessel was full until 34% of yeast slurry was removed at 24 hr (●).

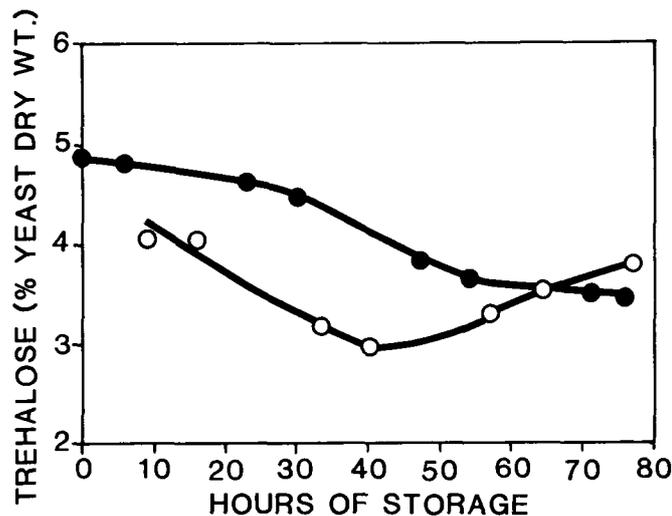


Fig. 2. Trehalose content of yeast during storage in a 200-bbl vessel. One vessel remained filled with yeast slurry over an 80-hr period (o), while the other vessel was full until 34% of yeast slurry was removed at 24 hr (●).

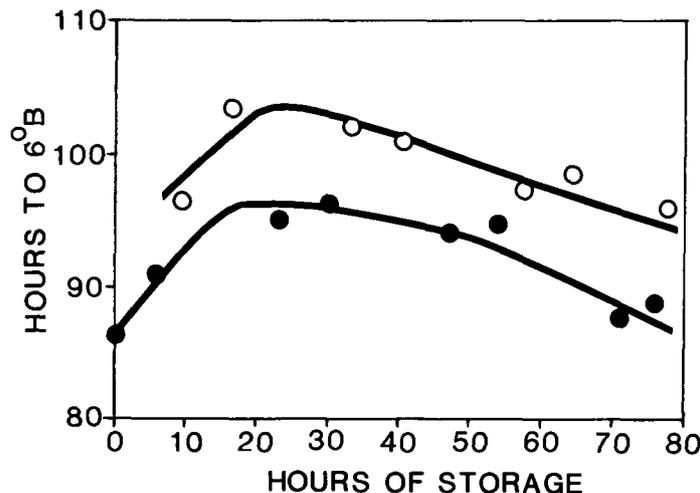


Fig. 3. Fermentation time (hours to 6°Balling) of yeast in a mini-fermentation after storage in either a full vessel (o) or a vessel that was full until 34% of yeast slurry was removed at 24 hr (●).

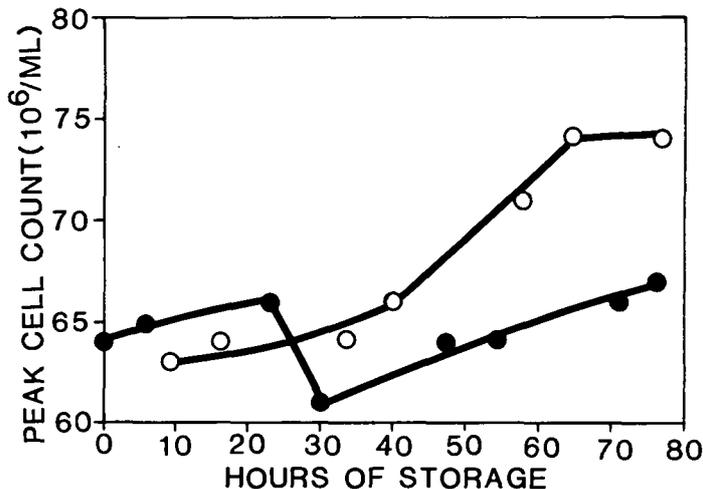


Fig. 4. Peak cell counts of yeast in a mini-fermentation after storage in either a full vessel (o) or a vessel that was full until 34% of yeast slurry was removed at 24 hr (●).

batches of yeast in mini-fermentations show that a production yeast with the same glycogen content (approximately 15%) can have different fermentation performances. The fermentation time of the yeast stored by either method increased over the first 30 hr of storage and thereafter steadily decreased (Fig. 3). The initial increase in fermentation time suggests that there was a period of metabolic adaptation to the cold storage conditions. In summary, although glycogen content was affected by yeast storage conditions (Fig. 1), this did not correlate with fermentation performance (Fig. 3).

In mini-fermentations of the yeast that had been stored in the full vessel, peak cell counts increased from 63 to 74×10^6 /ml over the first 60 hr (Fig. 4) and then remained constant for the next 20 hr. Fermentations of yeast stored in the partially filled vessel showed an initial rise in the peak cell counts, but declined when 34% of the yeast was removed at 24 hr. After this decline, the peak cell count steadily increased over the remaining storage period, but at a slower rate than in the full vessel. The increase in peak counts seen in both vessels could be caused by increased agitation efficiency, which would introduce more oxygen into the slurry. Oxygen is used by the yeast for the synthesis of fatty acids and sterols (3,8). Quain and co-workers (19) suggested that the energy source for lipid synthesis is yeast glycogen, and they showed that yeast sterol levels increase when stored in a stirred fermentor (18). However, in our study there was no correlation between glycogen decline and an increase in peak cell counts.

There are several reasons why no correlation existed between glycogen and yeast performance in this study. Our yeast contained lower levels (10–15%) of glycogen than yeasts used by other workers (30–40%). During a normal fermentation, the glycogen content of our yeast reached a maximum of 20–30% shortly after the cessation of yeast growth but declined toward the end of fermentation. Cantrell and Anderson (6) showed that low levels of glycogen (2.5%) did not impair fermentation performance in a production ale yeast. Therefore, there may not be a relationship between glycogen and yeast performance for all strains, particularly those with inherently low glycogen levels.

Another difference between this investigation and previous studies is the duration of yeast storage. Other workers (12) stored their yeast for five to six days and compared the stored yeast with the original. However, we carried out a detailed examination of yeast over three days, which is a normal period for yeast storage (14).

Finally, the conditions used by other workers to achieve different glycogen levels are likely to have also affected other metabolic processes. McCaig and Bendiak (11) noted that the viability of their yeast slurry declined to 70% after five days of stirring in a liter flask, indicating that yeast processes other than glycogen metabolism were being affected. In our study, the viability of yeast stored in both the full and the partially full vessels remained between 90 and 96%. Growth conditions can profoundly affect yeast performance, thus it is also conceivable that the yeast used by Quain and Tubb (17), grown on two different media, were changed in ways other than glycogen content. Therefore, although yeast glycogen content correlates with yeast performance during storage under harsh conditions, the effect may not be solely attributable to glycogen level, but to several other metabolic processes.

In this study we found that yeast glycogen levels did not correlate with yeast performance. If glycogen content is used as a criterion

for pitching yeast, as has been suggested (17), this could lead to over-pitching and inefficient yeast management. Our work and that of Cantrell and Anderson (6) indicates that all yeasts do not behave in the same manner. If glycogen is to be used as an indicator of yeast "fitness" in brewery operations, it must first be determined if glycogen measurement is meaningful. We monitored the glycogen content of 25 batches of production yeast and subsequent fermentation performance in mini-fermentations over one month. The results showed no significant relationship between fermentation performance and yeast glycogen content.

There is a need to determine the fitness of yeast before pitching. However, the evidence suggests that yeast fitness is not controlled by a single factor, but by multiple factors. Modern biochemical theories suggest that the performance of an organism or a pathway is not controlled by a single factor or enzyme (9). Indeed, the performance of an organism can only be accurately predicted from the summation of a large number of interacting factors. Therefore, a test for yeast performance that relies on a single characteristic will deviate from the predicted under certain conditions, whereas a test based on the whole organism should be more reliable.

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