Variables influencing viability of brewer’s yeast

Michael Nicholson and Brooke Pearson

Oxnard College

12/12/2012
Abstract

Home brewers use packaged brewer’s yeast (*Saccharomyces cerevisiae*) for the production of beer. Since yeast viability dramatically impacts fermentation, home brewers are concerned with the factors that might influence it. The purpose of this study was to investigate packaged yeast viability as influenced by yeast inoculum form (dry or liquid), age (fresh or 2-year old), and temperature (70°F, 100°F, and 130°F). Volumeters were used to measure CO₂ production of all possible combinations of treatments. Each volumeter contained 15 ml of yeast slurry (1x10⁸ cells/ml). Production of CO₂ was measured over approximately 17 hours, and the hourly average rate of CO₂ production established the data set. A factorial MANOVA supported the alternate hypothesis (Hₐ) that the three factors under study each contribute to differences in CO₂ production (all possible combinations, α = 1% significance), while individual one-way ANOVAs explored specific pairs of factors to elucidate subtle differences between groups. The fresh liquid yeast performed best, overall, at 70°F and 100°F, but failed at 130°F, while old liquid yeast performed worst, failing at all treatment temperatures. The fresh dry and old dry yeast performed at all temperature treatments, with fresh outperforming old consistently, and with better performance with decreased temperature treatments. These findings indicate that the home brewer, using packaged yeast, should definitely consider yeast format, age, and temperature when preparing to brew. Specifically, because fresh liquid yeast packages appear to be more sensitive to temperature and have a shorter shelf life than dry yeast packages, it is important to keep them cool and use them as soon as possible after purchasing them. Dry yeast packages may be better options for those who need a supply of yeast on-hand for use in the future, or for those who cannot keep the packages cool while transporting them after purchasing them.
Variables Influencing the Viability of Brewer’s Yeast

Brewer’s yeast (*Saccharomyces cerevisae*) is available to the non-commercial home brewer in a wide diversity of strains and inoculum forms. Dry yeast, available in small packets, is a trusted standard with good shelf-life and stability, but there are limited available varieties (Colby, 2006). Liquid yeast, on the other hand, available in tubes or large packets, offers greater diversity in terms of variety and strain availability, but has a shorter shelf-life (Lewis, 2007).

Home brewers must constantly balance the use of the two different yeast forms as they suit the particular needs of a specific brew (see sidebar for an overview of the brewing process). When using either form, however, home brewers always worry about the viability of their yeast. This is something that home brew shop owners are aware of as well, and some even offer ice packs to home brewers so the yeast can be transferred home safely without a decrease in yeast viability due to the potentially high temperatures in transit. The home brewer runs the risk of reduced yeast viability as a result of heating of the package, especially, for example, if the package is left in a hot car for a short time while errands are being attended to. Additionally, yeast longevity in the package is a wide-spread concern amongst home brewers, and a constant question is whether a particular package of yeast is “still good” after a certain date, relative to either a manufacturing date or an expiration date.

This study addresses the issue of yeast viability in terms of CO₂ production potential of different yeast samples subjected to different treatment conditions. Production of CO₂ under controlled conditions was used as an indicator of cellular viability of a package of yeast. Production of CO₂ was evaluated as influenced by yeast inoculum format (dry vs. liquid), age (old vs. new), and temperature treatment (70/100/130°F).
Materials and Methods

Yeast Sources

Packages of liquid and dry yeast were obtained from a local home brew store. Old and new packages of liquid yeast, Wyeast 1272 American Ale II yeast (Wyeast Laboratories, Inc., Odell, OR, 97044), were stamped with manufacture dates of 6 April 2010 and 26 June 2012, respectively. Old and new packages of dry yeast, Safale-US05 (Fermentis, Milwaukee, WI, 53214), were stamped with expiration dates of 02/2011 and 11/2013 respectively. The old packages of yeast were specifically purchased for this study in 2010 when it was still considered fresh, and was stored in refrigerated conditions. The new packages of yeast were purchased prior to experimental use. See Figure 1 for examples of packaged yeast.

Preparation of Liquid Yeast

The package of yeast was given time so that the yeast could settle (more than two years of settling for the old yeast; at least two days of settling for the new yeast). The supernatant was decanted off (≈ 60 ml) and then an equal volume of water replaced the supernatant (total volume ≈ 93 ml). Three equal samples of 30 ml each were prepared and then maintained for an hour at each of the three different treatment temperatures (70, 100, and 130°F). A cell count was established using a hemocytometer and the proper dilution ratio from the samples was determined to ensure an accurate cell count in the working viability test.

Preparation of Dry Yeast

The yeast package was divided into three roughly equal quantities (≈ 3.5 g) and each subsample was maintained for one hour at one of the three different treatment temperatures (70, 100, and 130°F). After treatment, each subsample was rehydrated in 30 ml of water according to the manufacturer directions. A cell count was established for each subsample/treatment using a
hemocytometer, and the proper dilution ratio for each was determined to ensure an accurate cell count in the working viability test.

**Standardization of Samples**

For each treatment studied, the samples were equivalently prepared such that each had 1x10^8 cells per ml (the standard pitching rate recommended for home brewing) and a sugar (brewer’s sugar, or D-glucose) concentration of 1.040 S.G. (the standard yeast starter sugar concentration recommended for home brewing). Each individual replicate consisted of 15 ml in total.

**Measurements of CO₂ Production**

Each treatment was replicated six times and CO₂ production was measured with a volumeter. Volumeters were prepared using 150 x 18 mm test tubes and modified inverted 10 ml disposable pipettes. The end of the pipette that normally inserts into a pipette pump was removed to increase the diameter of the opening, and this opening served to collect CO₂ from the bottom of the volumeter apparatus. The actual pipette tip, accordingly, pointed upward and was inserted into a 10 ml (green) pipette pump. The pipette pump served to establish a cap at the top of the pipette, and also controlled head-space so that the volumeters could be easily set and re-set. Volumeters are shown in Figure 2. Periodically the volumeters were reset. After the first hour, typically, all volumeters were reset; additionally, when any volumeter reached 5 ml, all volumeters were re-set reset. Re-setting of volumeters was performed as needed until the experiment was concluded. Volumes of CO₂ produced was always recorded for each replication, along with the elapsed time of production, every time the volumeters were re-set.
Results and Analysis

Average volumes of CO$_2$ produced (in terms ml/hr) for each treatment group is summarized in Table 1. The fresh liquid treatment performed best, overall, at 70˚F and 100˚F, but failed at 130˚F. The old liquid treatment performed worst, failing at all treatment temperatures. The fresh dry and old dry performed at all temperature treatments, with fresh outperforming old consistently, and with better performance with decreased temperature treatments.

Table 1. Average volumes of CO$_2$ produced (ml/hr) for all format/age/temperature treatment groups.

<table>
<thead>
<tr>
<th></th>
<th>Liquid (Wyeast 1272)</th>
<th>Dry (Safale-US-05)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fresh</td>
<td>Old</td>
</tr>
<tr>
<td>70˚F</td>
<td>2.81</td>
<td>0.00</td>
</tr>
<tr>
<td>100˚F</td>
<td>2.25</td>
<td>0.00</td>
</tr>
<tr>
<td>130˚F</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

A multiple analysis of variance identified significant differences across all combinations of variables at the 1% significance level (see Table 2). Individual analyses of variance between pairs of treatment groups further elucidated significant differences (see Table 3 for summary statistics). The analyses indicate that the overall best performing yeast group was the new liquid yeast, while the overall worst performing yeast group was the old liquid yeast, which failed to perform at all. The liquid fresh yeast performed better than any other format and age combination, although the dry yeast, overall, performed significantly better than the liquid yeast, most likely due to the failure of the liquid old yeast to ferment. In general, yeast treated at 70˚F performed better than yeast treated at higher temperatures, regardless of the format or age, with the exception of the liquid old yeast, which, as noted, failed to perform at all treatment temperatures. See Figure 3 for a graphical presentation of idealized performance comparisons between treatments.
Table 2.
*Three-factor design Multiple Analysis of Variance*

A = Format  
B = Age  
C = Temperature

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Degrees of Freedom</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F</th>
<th>F-crit</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replication</td>
<td>5</td>
<td>0.07</td>
<td>0.01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Main (A)</td>
<td>1</td>
<td>2.19</td>
<td>2.19</td>
<td>158.40</td>
<td>16.258</td>
<td>0.01 significant</td>
</tr>
<tr>
<td>Error (A)</td>
<td>5</td>
<td>0.07</td>
<td>0.01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sub (B)</td>
<td>1</td>
<td>18.79</td>
<td>18.79</td>
<td>1214.74</td>
<td>10.044</td>
<td>0.01 significant</td>
</tr>
<tr>
<td>AxB</td>
<td>1</td>
<td>7.94</td>
<td>7.94</td>
<td>513.23</td>
<td>10.044</td>
<td>0.01 significant</td>
</tr>
<tr>
<td>Error (B)</td>
<td>10</td>
<td>0.15</td>
<td>0.02</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sub-sub (C)</td>
<td>2</td>
<td>10.59</td>
<td>5.30</td>
<td>852.64</td>
<td>5.179</td>
<td>0.01 significant</td>
</tr>
<tr>
<td>AxC</td>
<td>2</td>
<td>3.60</td>
<td>1.80</td>
<td>289.86</td>
<td>5.179</td>
<td>0.01 significant</td>
</tr>
<tr>
<td>BxC</td>
<td>2</td>
<td>7.48</td>
<td>3.74</td>
<td>602.06</td>
<td>5.179</td>
<td>0.01 significant</td>
</tr>
<tr>
<td>AxBxC</td>
<td>2</td>
<td>5.84</td>
<td>2.92</td>
<td>470.36</td>
<td>5.179</td>
<td>0.01 significant</td>
</tr>
<tr>
<td>Error (C)</td>
<td>40</td>
<td>0.25</td>
<td>0.01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>71</td>
<td>56.97</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Coefficient of Variation (A) 11.55%  
Coefficient of Variation (B) 12.23%  
Coefficient of Variation (C) 7.75%

Table 3.
*Summary of individual Analysis of Variance comparisons significant at 1% or 5%.*

<table>
<thead>
<tr>
<th>Main Factor</th>
<th>Comparison</th>
<th>F calc.</th>
<th>F crit.</th>
<th>Significance Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry Fresh</td>
<td>70°F vs 100°F</td>
<td>9.77</td>
<td>4.97</td>
<td>0.05</td>
</tr>
<tr>
<td>Dry Fresh</td>
<td>70°F vs 130°F</td>
<td>481.21</td>
<td>10.04</td>
<td>0.01</td>
</tr>
<tr>
<td>Dry Old</td>
<td>70°F vs 100°F</td>
<td>6.20</td>
<td>4.97</td>
<td>0.05</td>
</tr>
<tr>
<td>Dry Old</td>
<td>70°F vs 130°F</td>
<td>87.10</td>
<td>10.04</td>
<td>0.01</td>
</tr>
<tr>
<td>Liquid Fresh</td>
<td>70°F vs 100°F</td>
<td>20.16</td>
<td>10.04</td>
<td>0.01</td>
</tr>
<tr>
<td>Liquid Fresh</td>
<td>70°F vs 130°F</td>
<td>1600.60</td>
<td>10.04</td>
<td>0.01</td>
</tr>
<tr>
<td>Overall</td>
<td>Liquid Fresh vs. Dry Fresh</td>
<td>32.19</td>
<td>10.04</td>
<td>0.01</td>
</tr>
<tr>
<td>Overall</td>
<td>Liquid Fresh vs. Dry Old</td>
<td>145.18</td>
<td>10.04</td>
<td>0.01</td>
</tr>
<tr>
<td>70°F</td>
<td>Liquid Fresh vs. Dry</td>
<td>309.78</td>
<td>10.04</td>
<td>0.01</td>
</tr>
<tr>
<td>Overall</td>
<td>Dry Fresh vs. Old</td>
<td>541.83</td>
<td>10.04</td>
<td>0.01</td>
</tr>
<tr>
<td>70°F</td>
<td>Dry Fresh vs. Old</td>
<td>439.55</td>
<td>10.04</td>
<td>0.01</td>
</tr>
<tr>
<td>130°F</td>
<td>Dry Fresh vs. Old</td>
<td>70.02</td>
<td>10.04</td>
<td>0.01</td>
</tr>
</tbody>
</table>

**Conclusions**

The new liquid yeast outperforms all other combinations at 70°F and 100°F but did not perform at 130°F. The old liquid yeast completely failed at all temperatures. From this, it can be concluded that liquid yeast loses viability over time and is highly sensitive to higher
temperatures. New and old dry yeast performed at all temperature treatments and performed better with decreasing temperatures; the new yeast performed consistently better than the old. From this, it can be concluded that the dry yeast is less sensitive to both temperature and storage duration. Taken as a whole, the data appears to indicate that dry yeast performance is not as likely to be impacted by storage duration or treatment as liquid yeast performance is. However, if storage duration is short and storage treatment is favorable, the performance of liquid yeast is likely to be better than the performance of dry yeast.

Discussion

The findings of this study indicate that temperature is an important factor to consider when handling yeast, and that liquid yeast, in particular, is much more sensitive to higher temperatures than dry yeast. Furthermore, age of the yeast package factors in as a strong influence, such that old liquid yeast may completely fail, whereas old dry yeast may simply demonstrate reduced viability and CO₂ production potential. These results are consistent with other reports addressing yeast viability relative to age and storage (Morimura, Hino, & Kida, 1998; Powell, Quain, & Smart, 2003). All of this is of practical significance to the home brewer, and underscores the importance of keeping yeast packages cool during transit from the homebrew shop (it is also important to make certain that purchases of liquid yeast are appropriately cooled during transit from distant vendors). Temperature is of less importance when it comes to dry yeast, but should remain a factor of consideration.

These findings also provide home brewers with insights regarding the limits of ages for liquid and dry yeast. In specific, liquid yeast older than two years is probably not worth using for brewing purposes. On the other hand, if two year old dry yeast is all that is available, it appears likely that it may perform adequately, provided it was kept cool the entire time.
Production of CO₂, as measured for this study, correlates with yeast viability in that the fermentation performance of a culture of yeast is strongly related to the CO₂ byproduct of the fermentation process. As such, measuring CO₂ production as an indicator of viability is an indirect observation approach and it ignores a wide array of other yeast performance attributes (e.g., attenuation, diacetyl reabsorption, production of off-flavors, flocculation, etc.). One of the caveats of this study is that all other factors have been ignored in an effort to produce a simple, easily understood, easily quantified, estimate of viability. The strength of using CO₂ production to estimate yeast viability is that anyone should be able to easily replicate the work, including the interested home brewer, provided that the right equipment is available.

In the preliminary phases of this study, standard Durham Tubes were used as volumeters to quantify CO₂ production (see Figure 4). The tubes held about 25 ml of yeast slurry and allowed for 5 ml of CO₂ production to be quantified before needing to be re-set. Variance between replicates was found to be very high, and efforts to reduce variance were met with poor success. The modified volumeter used in this study was determined to be the most appropriate for the experimental conditions under observation. Several designs were considered before settling on the configuration that was finally employed, consisting of a 10 ml pipette, 150 mm x 18 mm test tube, and a pipette pump (see Figure 2).

Although other methods of viability testing exist and can be employed in the laboratory (Bouix & Leveau, 2001; Boyd et al., 2003; Sami, Ikeda, & Yabuuchi, 1994; Stewart & Russell, 1998; Trevors, Merrick, Russell, & Stewart, 1983), it is clear that there is no truly simple method that can easily be implemented by the standard home brewer (see sidebar for an overview of viability testing alternatives). Equipment limitations (not expensive or complicated) and limitations of the actual methods (e.g., methylene blue is easy but requires a high actual viability
for reliable results) were considered as the experimental protocol was developed. With the
general understanding gained from the results of this study, more focused investigations are
warranted. Future work with similar CO$_2$ volumeters might assess the nuances of age of the
yeast package (an array of ages, for example, instead of only fresh and “old”) or deeper
interactions between age and temperature as they relate to performance. All of this work could
feasibly be done by home brewing enthusiasts, and it is hoped that some of the results would find
their way back to the scientific community at large, to enrich our understanding of the variables
that influence yeast viability for home brewing purposes.
References


Figure 1. 
*Examples of packaged yeast, similar to what was used for the study.*
Figure 2.
*Volumeters used to measure volumes of CO$_2$ produced by yeast treatments.*
Figure 3. Idealized performance comparisons between yeast treatments.

**CO₂ Production of Yeast as Influenced by Experimental Factors**

- **Yeast Form, Age, and Temperature**
- **CO₂ Produced (in ml)**
- **Elapsed Time (in hrs)**

- Liquid Fresh - 70°F
- Dry Fresh - 70°F
- Dry Old - 70°F
- Liquid Fresh - 100°F
- Dry Fresh - 100°F
- Dry Old - 100°F
- Liquid Fresh - 130°F
- Dry Fresh - 130°F
- Dry Old - 130°F
Figure 4.
Graduated Durham Tube for fermentation studies.