Variables Influencing Viability of Brewer's Yeast

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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 CO_2 production of all possible combinations of treatments. Each volumeter contained 15 ml of yeast slurry ($1x10^8$ cells/ml). Production of CO_2 was measured over approximately 17 hours, and the hourly average rate of CO_2 production established the data set. A factorial MANOVA supported the alternate hypothesis (H_A) that the three factors under study each contribute to differences in CO_2 production (all possible combinations, a = 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.

Brewer's yeast (*Saccharomyces cerevisae*) is available to the 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).

Figure 1. Examples of packaged yeast, similar to what was used for the study.

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 seeks to address the issue of yeast viability in terms of CO_2 production potential of different yeast samples subjected to different treatment conditions. Production of CO_2 under controlled conditions will be used as an indicator of cellular viability of a package of yeast. Production of CO_2 will be evaluated as influenced by yeast inoculum format (dry vs. liquid), age (old vs. new), and temperature treatment (70/100/130˚F).

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The Brewing Process

Brewing begins with a "mash" of malted barley. Malted barley is allowed to sprout (activating enzymes), and is then kiln dried. The kilning temperature determines the degree of coloration, among other things, in the final malt. Mash consists of malted barley and water at a temperature of about 150˚F; the starches in the grain are broken down into maltose sugars, thanks to the enzymes in the malted barley itself. Once the starches are completely converted into sugars, the sugars become soluble in the water. The mash can also contain other malted or unmalted grain (e.g. wheat or rye), as well as other sources of starch and sugar (e.g. pumpkin or sweet potato).

The sugary liquid is then separated from the grain in a process called "sparging." The sparge is collected in a large kettle to produce something called "wort," which will ultimately be fermented into beer. The wort is boiled, usually for about 90 minutes, to reduce it and concentrate the sugars. During the boil, hops can be added to impart bitter and aromatic compounds. A diversity of hops is available, with some that are primarily used for bittering, others for aroma, and some for dual purposes. During the boil, other flavoring adjuncts, as well as additional forms of sugar, may be added.

When the boil is complete, the finished wort is cooled and transferred to the fermentation tank. Yeast is added to the fermentation tank, to metabolize the sugars, producing alcohol and other fermentation byproducts in the process. Carbon dioxide is a fermentation byproduct, and it can be used to naturally carbonate beer in the bottle, provided that live yeast and fermentable sugar are both present in the bottle itself.

While many factors enter into the entire brewing equation, the last is the yeast itself, responsible for the actual conversion of sugars to alcohol. Different yeasts have slightly different fermentation profiles, producing different compounds that contribute to the different established styles of beer. Of principal concern to the brewer is the health of the yeast to be used and its ability to ferment. Thus, yeast viability is an important consideration, given that so much time, energy, and materials are invested in a beer by the time that the yeast is added.

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 they were 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.

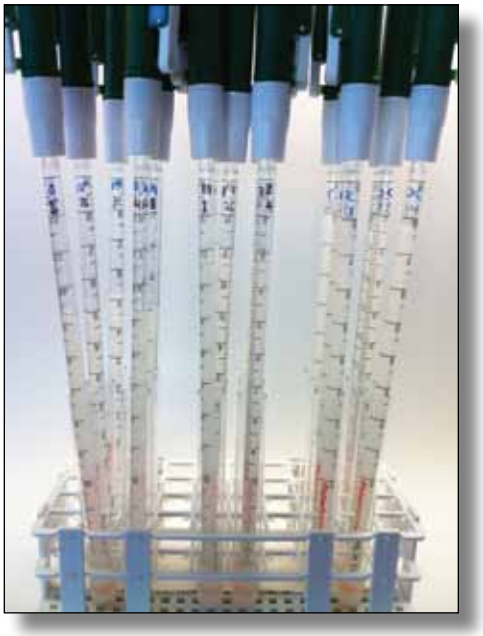

Figure 2. Volumeters used to measure volumes of CO_2 produced by yeast treatments.

Table 1. Average volumes of CO_2 produced (ml/hr) for all format/age/temperature treatment groups.

	Liquid (Wyeast 1272)		Dry (Safale-US-05)	
	Fresh	Old	Fresh	Old
70˚F	2.81	0.00	1.55	1.12
100˚F	2.25	0.00	1.47	1.07
130˚F	0.00	0.00	1.10	0.84

Measurements of CO_2 Production

Each treatment was replicated six times and CO_2 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 CO_2 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 reset. 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 reset. Re-setting of volumeters was performed as needed until the experiment was concluded. The volume of CO_2 produced was recorded for each replication, along with the elapsed time of production, each time the volumeters were reset.

Results and Analysis

Average volumes of CO_2 produced (in terms of 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.

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.

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 very 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 duration of storage. Taken as a whole, the data appear to indicate that dry yeast performance is not as likely to be impacted by storage

Table 2. Three-factor design Multiple Analysis of Variance

A = Format, B = Age, C = Temperature							
Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F	F-crit	Significance	
Replication	5	0.07	0.01				
Main (A)	1	2.19	2.19	158.40	16.258	0.01	significant
Error (A)	5	0.07	0.01				
Sub (B)	1	18.79	18.79	1214.74	10.044	0.01	significant
AxB	1	7.94	7.94	513.23	10.044	0.01	significant
Error (B)	10	0.15	0.02				
Sub-sub (C)	2	10.59	5.30	852.64	5.179	0.01	significant
AxC	2	3.60	1.80	289.86	5.179	0.01	significant
BxC	2	7.48	3.74	602.06	5.179	0.01	significant
AxBxC	2	5.84	2.92	470.36	5.179	0.01	significant
Error (C)	40	0.25	0.01				
Total	71	56.97					
Coefficient of Variation – (A) 11.55%, Coefficient of Variation – (B) 12.23%, Coefficient of Variation – (C) 7.75%							

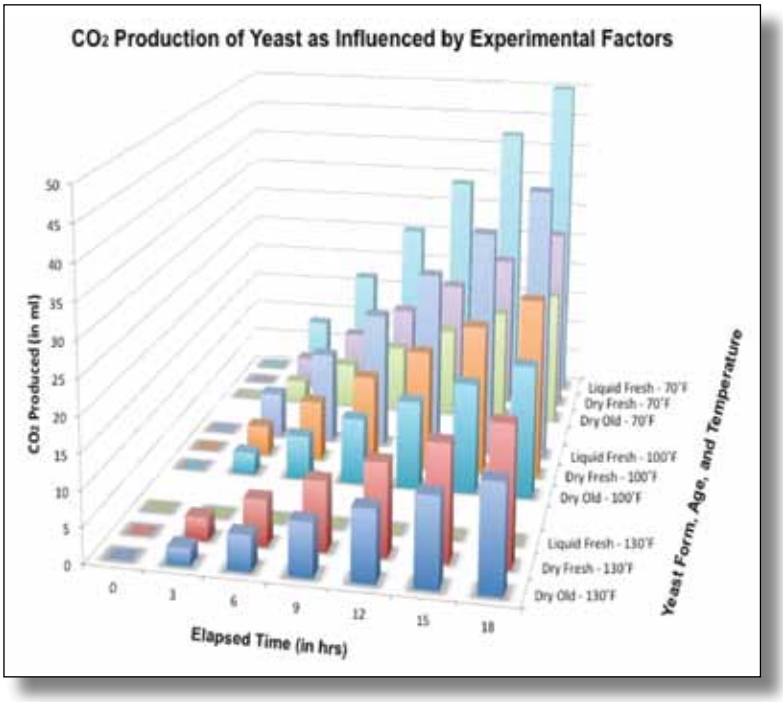

Figure 3. Idealized performance comparisons between yeast treatments.

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

Main Factor	Comparison	F calc.	F crit.	Significance Level
Dry Fresh	70˚F vs 100˚F	9.77	4.97	0.05
Dry Fresh	70˚F vs 130˚F	481.21	10.04	0.01
Dry Old	70˚F vs 100˚F	6.20	4.97	0.05
Dry Old	70˚F vs 130˚F	87.10	10.04	0.01
Liquid Fresh	70˚F vs 100˚F	20.16	10.04	0.01
Liquid Fresh	70˚F vs 130˚F	1600.60	10.04	0.01
Overall	Liquid Fresh vs. Dry Fresh	32.19	10.04	0.01
Overall	Liquid Fresh vs. Dry Old	145.18	10.04	0.01
70˚F	Liquid Fresh vs. Dry	309.78	10.04	0.01
Overall	Dry Fresh vs. Old	541.83	10.04	0.01
70˚F	Dry Fresh vs. Old	439.55	10.04	0.01
130˚F	Dry Fresh vs. Old	70.02	10.04	0.01

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.

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 is a strong influence, such that old liquid yeast may completely fail, whereas old dry yeast may simply demonstrate reduced viability and CO_2 production potential. These results are consistent with other reports addressing yeast viability relative to age and storage (Morimura et al., 1998; Powell et al., 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 CO_2, as measured for this study, correlates with yeast viability in that the fermentation performance of a culture of yeast is strongly related to the CO_2 byproduct of the fermentation process. As such, measuring CO_2 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 CO_2 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 CO_2 production (Figure 4). The tubes held about 25 ml of yeast slurry and allowed for 5 ml of CO_2 production to be quantified before needing to be reset.

Figure 4. Graduated Durham Tube for fermentation studies.

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 (Figure 2).

Although other methods of viability testing exist and can be employed in the laboratory (Bouix and Leveau, 2001; Boyd et al., 2003; Sami et al., 1994; Stewart and Russell, 1998; Trevors et al., 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 evaluation 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 facilitate assessment of 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 Cited

Bouix, M., and J. Leveau. 2001. Rapid assessment of yeast viability and yeast vitality during alcoholic fermentation. *Journal of the Institute of Brewing* 107(4): 217-225.

Boyd, A., T. Gunasekera, P. Attfield, K. Simic, S. Vincent, and D. Veal. 2003. A flow-cytometric method for determination of yeast viability and cell number in a brewery. *FEMS Yeast Research* 3: 11-16.

Colby, C. 2006. Dried brewing yeast on the rise. *Brew Your Own* Jan/Feb 30-31.

Lewis, A. 2007. *The Home Brewer's Answer Book: Solutions to Every Problem, Answers to Every Question*. North Adams, MA: Storey Publishing.

Morimura, S., T. Hino, and K. Kida. 1998. Storage of pitching yeast for production of whisky. *Journal of the Institute of Brewing* 104: 213-216.

Powell, C., D. Quain, and K. Smart. 2003. The impact of yeast cell age on fermentation, attenuation, and flocculation. Pp. 272-280, In: K. Smart (Ed.), *Brewing Yeast Fermentation Performance* (2nd ed.). Oxford, UK: Blackwell Science.

Sami, M., M. Ikeda, and S. Yabuuchi. 1994. Evaluation of the alkaline methylene blue staining method for yeast activity determination. *Journal of Fermentation and Bioengineering* 78(3): 212-216.

Stewart, G., and I. Russell. 1998. *An Introduction to Brewing Science & Technology. Series III: Brewer's Yeast*. London, England: The Institute of Brewing.

Trevors, J., R. Merrick, R. Russell, and G. Stewart. 1983. A comparison of methods for assessing yeast viability. *Biotechnology Letters* 5(2):131-134.

An overview of methods to evaluate yeast viability.

Yeast viability is often evaluated in terms of numbers of living cells in a particular culture or inoculum source. Viability results can be use to understand the overall health of the yeast, and to guide decisions regarding use of the yeast for brewing, sub-culturing, further analysis, etc.

Plating and counting is perhaps the least costly viability evaluation method. This method directly measures numbers of live cells and is very inexpensive, but it requires time for cultures to grow on the plates. Though it is accurate if used properly, the relevance of the count is relative to the date the sample was measured.

Methylene blue staining is a fast, low-cost approach that requires the use of a microscope, hemocytometer, and the methylene blue dye. As with other staining procedures, this approach differentiates live and dead cells; the living cells remain colorless, while dead cells become stained blue. The yeast being evaluated with methylene blue, must be fairly fresh, and must be examined within a short time after being stained. Furthermore, results become unreliable as actual yeast viability drops below 90%. As such, for regularly testing viability, this method is simple and easy to implement. However, when attempting to evaluate yeast with completely unknown viability, or yeast cultures that are not quite fresh, this method is not appropriate.

CO_2 production assaying, as described in this paper, is a moderately time-consuming, but low-cost, approach that requires a microscope, hemocytometer, and volumeters or Durham Tubes. CO_2 production provides an estimate of viability based on indirect data from known numbers of cells (counted microscopically). Results can be obtained in hours, but factors influencing fermentation (temperature, starting numbers of cells, sugar concentration of medium, etc.) must be held constant between tests to ensure reproducibility and relevance of results between samples.

Fluorometric assaying is a fast, high-cost approach, requiring a microscope capable of epifluorescence, special fluorescent dyes, and a hemocytometer. This method is highly reliable, and directly and easily differentiates live from dead cells by color indicators. Epicocconone, for example, is a pH dependent fluorescent dye that fluoresces orange in live cells and green in dead cells. The contrast and brilliance allow for easy determination of viable cell numbers. Automatic fluorescence assaying is possible, though potentially expensive (e.g. with a Cellometer).

Dielectrophoresis efficiently separates non-viable and viable yeast cells in a mixture. Positive and negative dielectrophoretic forces generated by microelectrodes in a small chamber are used to selectively isolate viable and nonviable yeast cells. This method is expensive, due to the equipment needed to separate the yeast, but is quick. Actual quantification of the yeast must be accomplished separately, or as a secondary process, using any of the previously noted methods or assays. The quantification steps may slow down the evaluation process.

Flow cytometry is perhaps the most expensive method, though it is also the fastest and most reliable. This method uses fluorescence and a cell separating or counting unit, essentially operating dielectrophoretically. A laser is used to enumerate stained cells as they pass through a cell sorter that separates non-viable and viable cells in real time. Usually, viable cells retain the dye and fluoresce as they move through the sorter, and are thus easily separated and counted. This method has a very low error rate, and delivers results very quickly, but requires expensive equipment and technical support.