

Introduction

The Craft Brewing Industry

- The number of craft breweries have increased 5-fold over the past two decades.
- 14.6% of all beer spending is on craft brews (Brewer's Association).
- New flavor profiles and alcohol content provide countless varieties of beer.

Pitching in Brewing

- Pitching: yeast is added to wort to start the fermentation process.
- Brewers use a starter culture for their first batch of beer.
 - The culture is then re-pitched into the next fermentor to start the next batch.
- Alcohol is the byproduct of the fermentation process.
 - A re-pitched culture will contain alcohol that was produced during the previous fermentation process.
 - This amount will vary from beer to beer.
- Microorganisms, including yeast, are typically killed by alcohol.
- Yeast have artificially evolved to withstand different levels of alcohol.
 - In order to change the alcohol content of beer, yeast needs to be able to withstand the brewing conditions.

Growth Curve Analysis

- Fermentation requires an adequate number of healthy yeast cells to be re-pitched from batch to batch.
- The health of yeast can be analyzed through how quickly the cells grow.
- This can be determined by looking at the growth rate in the mid-log phase or how long the growth process takes to start to grow.

Medium Tolerance (5-10%)

- Hefeweizen Ale Yeast (WLP300) | Used to brew Weissbier and Weizenbock
- German/Kölsch Ale Yeast (WLP029) | Used to brew Altbier, California Common, Cream Ale, and Kölsch
- Belgian Ale Yeast (WLP550) | Used to brew Belgian Dark Strong Ale, Belgian Dubbel, Belgian Tripel, Saison, and Witbier
- Belgian Wit Ale Yeast (WLP400) | Used to brew Belgian Pale Ale, Belgian Tripel, Cider, Saison, and Witbier
- Belgian Saison I Ale Yeast (WLP565) | Used to brew Belgian Dubbel, Belgian Pale Ale, Belgian Tripel, Saison, and Witbier
- Dusseldorf Alt Ale Yeast (WLP036) | Used to brew Altbier, Cream Ale, Kölsch, and Red Ale

Medium-High Tolerance (8-12%)

- Dry English Ale Yeast (WLP007) | Used to brew American IPA, Barleywine, Blonde Ale, Cider, Double IPA, Dry Mead, English Bitter, English IPA, Gin, Hazy/Juicy IPA, Imperial Stout, Old Ale, Pale Ale, Porter, Red Ale, Sake, Scotch Ale, Stout, Sweet Mead, Vodka, and Whiskey
- Saccharomyces brux-like Trois* (WLP644) | Used to brew American IPA, Blonde Ale, Double IPA, Hazy/Juicy IPA, Pale Ale, and Wild Specialty Beer

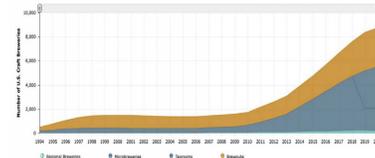
High Tolerance (10-15%)

- California Ale Yeast (WLP001) | Used to brew American IPA, American Wheat Beer, Barleywine, Blonde Ale, Brown Ale, California Common, Cider, Double IPA, Dry Mead, Imperial Stout, Old Ale, Pale Ale, Porter, Red Ale, Stout, and Sweet Mead
- San Diego Super Ale Yeast (WLP090) | Used to brew American IPA, Barleywine, Blonde Ale, Cider, Double IPA, Dry Mead, Imperial Stout, Old Ale, Pale Ale, Porter, Red Ale, Stout, and Sweet Mead
- Monastery Ale Yeast (WLP500) | Used to brew Belgian Dark Strong Ale, Belgian Dubbel, Belgian Pale Ale, Belgian Tripel, Biere de Garde, and Cider

Very High Tolerance (15%+)

- English Cider Yeast (WLP775) | Used to brew cider

U.S. Craft Brewery Count by Category



Objective

Analyze alcohol tolerance of different strains of *Saccharomyces cerevisiae* through growth curve analysis and compare this data to that of the claims made by commercial strain companies.

Hypothesis

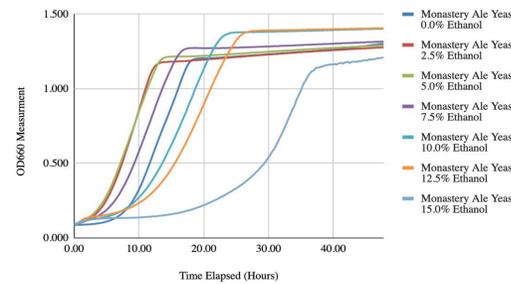
As alcohol levels increase, growth rates will decrease but some strains will be able to tolerate the high rates of alcohol.

Results

Yeast growth can be assessed using 96-well plates with the Multiskan Go Plate Reader

Sample growth curve from one of the twelve strains examined

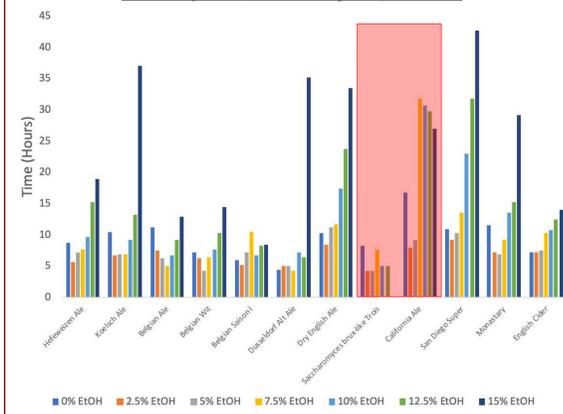
Monastery Ale Yeast Growth Curve



- 10 of the 12 strains examined showed growth curves similar to the monastery ale yeast depicted with varying degrees of growth (see below).
 - California Ale Yeast and *Saccharomyces brux-like Trois* showed inconsistent results (see red boxes in figures below).
 - Dusseldorf Alt Ale Yeast failed to grow at 15% EtOH conditions.

Some yeast strains require a longer time to acclimatize to increasing levels of alcohol whereas others are largely unaffected as indicated by the time in lag phase.

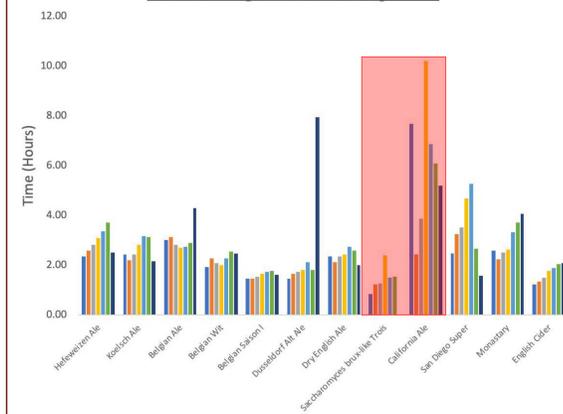
Time Required to Reach Log Phase Growth



- English Cider, Belgian Ale, Belgian Wit, and Belgian Saison I yeast were least affected by increasing levels of alcohol indicating a higher tolerance for these strains.
- San Diego Super was most affected by increasing levels of alcohol indicating a lower tolerance for this strain.
- Dusseldorf Alt Ale yeast was largely unaffected except under very high (15%) EtOH levels where it failed to grow entirely.

Increasing levels of alcohol affect growth rates for some strains, while not as much for other strains.

Cell Doubling Time at Mid Log Phase



- Growth rate tends to decrease (doubling time increases) as EtOH levels increase, but only mildly.
- Belgian Saison I Ale yeast was least affected by changes in alcohol levels.
- Belgian Ale yeast and Dusseldorf Alt yeast showed consistent growth except for at 15% EtOH where there was a significant drop off.

Conclusions

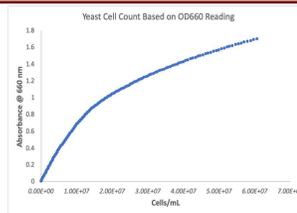
- The Multiskan Go Plate Reader provides reliable and consistent growth curve data that saves valuable time and resources.
- English Cider, Belgian Wit, and Belgian Saison I yeast are least affected by increasing levels of alcohol as indicated by both time in lag phase and by growth rate, despite the latter three strains considered only medium tolerant strains.
- Surprisingly, San Diego Super Ale and Monastery Ale appeared most affected by alcohol despite claims that they are higher alcohol tolerant strains.

Future Directions

- Repeat these experiments to corroborate the results.
- Determine what percentage of ethanol is ideal for each strain.
- Visualize the yeast under the microscope at various time-points to assess their health.
- Use re-pitching practices to analyze the effect of added alcohol over time.
- Determine if the age of a sample plays a role in the growth rate under alcohol conditions.

Materials and Methods

Yeast cell counts can be measured via Optical Density (OD660) readings.



Growth curves are an indicator of yeast health

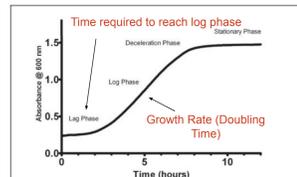
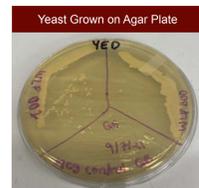


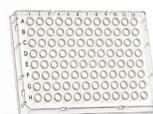
Figure 2. Typical Yeast Growth Curve. *Saccharomyces cerevisiae* grown in YPD media at 30°C for 12 hours with data measurements every 2 minutes.

12 strains are isolated and grown on YPD @ 30°C for 48 hours.



Colonies are selected for each strain and used to inoculate O/N cultures.

Each culture is diluted to an OD660 = 0.1 in 200 ul YPD w/ 0-15% EtOH and samples placed into 96 well plate.



96-Well Plate
Each well on the plate is filled with 200 uL of sample/ethanol

Plate is loaded in plate reader and OD660 is measured every 15 minutes for 48 hours to determine growth curves for varying conditions.



Thermo Scientific Multiskan Go Plate Reader
Parameters:
-30° C with continuous shaking at low speed
-1% absorbance readings taken at OD660
-One reading taken every 15 minutes

Growth curves are analyzed to determine yeast health and acclimatization as indicated by time in lag phase and growth rate.

Citations

- Market Development Committee. (2020, October 7). *Craft is vital to beer selection*. Brewers Association. <https://www.brewersassociation.org/brewing-industry-updates/craft-is-vital-to-beer-selection/>
- Whitelabs. (n.d.). *Yeast and bacteria bank: White Labs*. Whitelabs. <https://www.whitelabs.com/yeast-bank>

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