YEAST HEALTH AND VIABILITY IN HIGH GRAVITY AND SOUR BEERS

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BIG BEERS Belgians & Barleywines festival

Common Stresses that Negatively Effect Yeast Viability

Yeast reacts to stress by upregulating stress proteins, which there are thousands

Osmotic Stress => Ethanol Stress

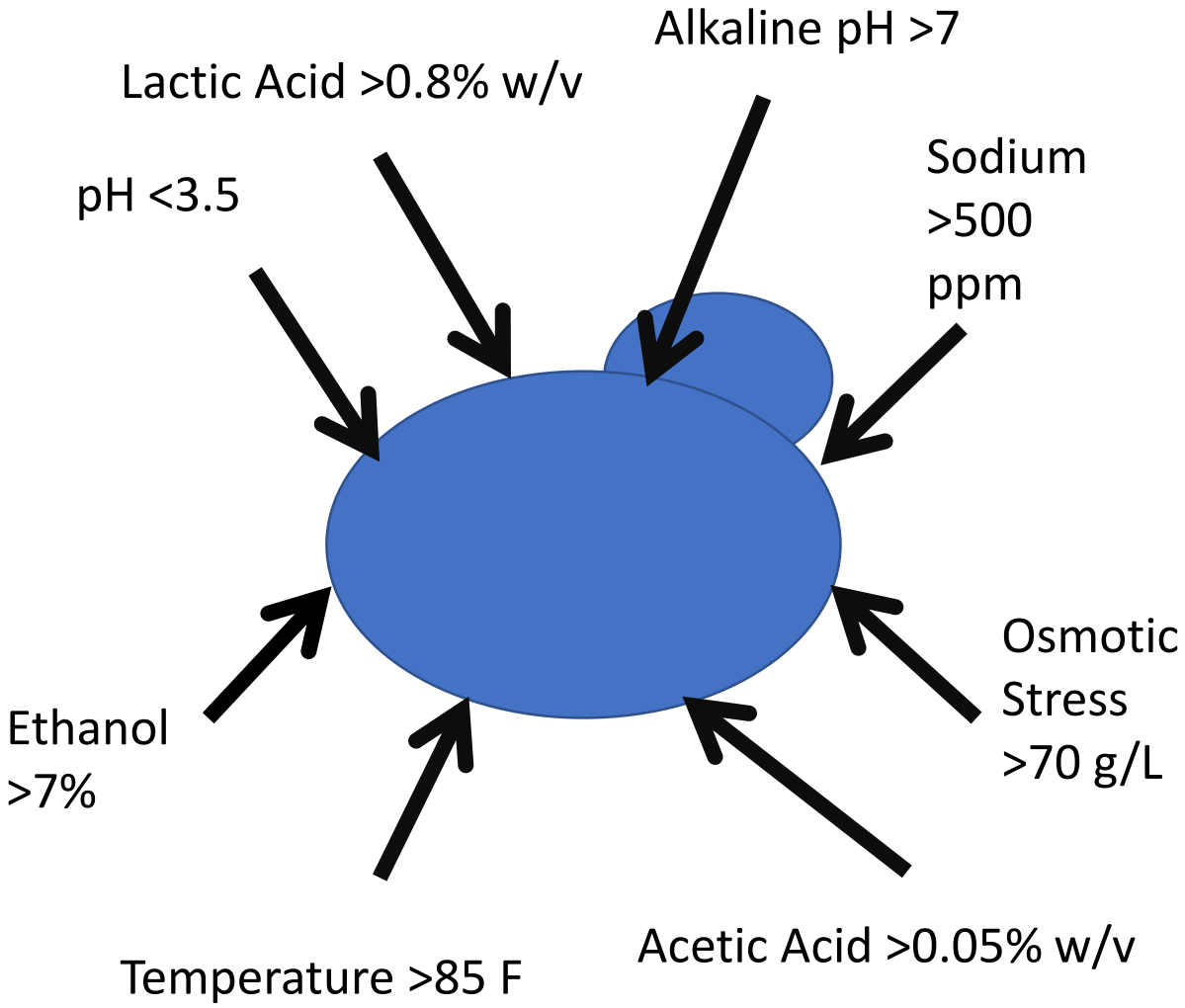
Osmotic Stress : Increase in glycerol production before fermentation leads to lag

Ethanol Stress : Inhibition of cell division, altered metabolism, cell structure and membrane function

pH stress : Yeast grows and ferments better in acidic conditions than neutral or alkaline

Low pH : Decreased membrane permeability, altered gene expression, loss of electrochemical gradient

Cell Stress is Synergistic! What about high gravity sour beers?!!



BIG BEERS Belgians & Barleywines festival

Ethanol Stresses that Negatively Effect Yeast Viability

Cell Viability and Growth

Metabolism

Cell Structure and Membrane Function

Table 1 Some effects of ethanol on yeast physiology

Cell function and ethanol influence	Source
Cell viability and growth	
Inhibition of growth, cell division and cell viability	Stanley *et al.* (1997)
Decrease in cell volume	Birch and Walker (2000)
Metabolism	
Lowered mRNA and protein levels	Chandler *et al.* (2004), Hu *et al.* (2007)
Protein denaturation and reduced glycolytic enzyme activity	Hallsworth *et al.* (1998)
Induction of heat shock proteins and other stress response proteins	Plesset *et al.* (1982)
Intracellular trehalose accumulation	Lucero *et al.* (2000)
Cell structure and membrane function	
Altered vacuole morphology	Meaden *et al.* (1999)
Inhibition of endocytosis	Lucero *et al.* (2000)
Increased unsaturated/ saturated fatty acid ratio in membranes	Alexandre *et al.* (1994)
Increase in ergosterol content of membranes	Sajbidor *et al.* (1995)
Loss of electrochemical gradients and proton-motive force	Petrov and Okorokov (1990)
Inhibition of transport processes	Leao and van Uden (1984)
Inhibition of H^+-ATPase activity	Cartwright *et al.* (1986)
Increased membrane fluidity	Mishra and Prasad (1989)

Yeast Health and Viability in Ultra High Gravity Brewing

Dan Strevey

Director of Quality

Big Beers, Belgians, and Barleywines 1/12/19

Fimbulvvinter

- Rum barrel aged Belgian style quintuple
- Bottled 11/30/17, and is tasting great!
- 16.9% ABV

Ultra High Gravity (UHG) Yeast Planning

- UHG wort typically >25°Plato (P),
- Strains of Success = alcohol tolerance and high attenuation!

Disclaimer, all our yeasts have been isolated and chosen for big beers over many tanks and many years. What works for us may not work for you purchased strains

- Chico – Can work, however above 25°P acetaldehyde can become a big concern.
- London Ale – Good alcohol tolerance, but variable apparent extract (AE)
- Trappist High Gravity – Very successful and predictable strain.
- Many other options out there.

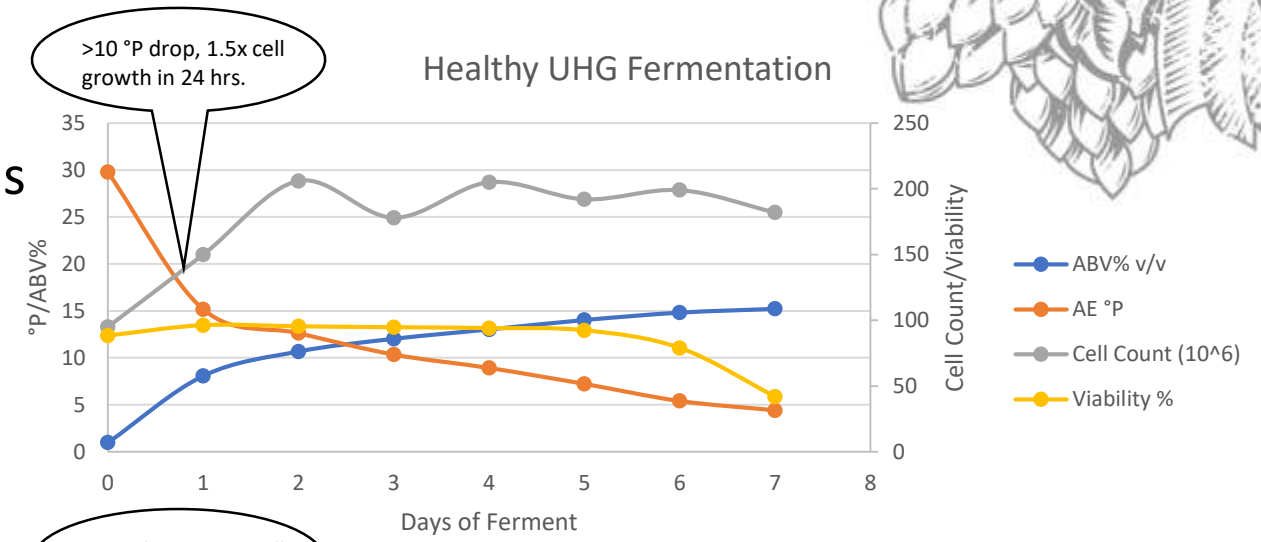
- Yeast Management
  - Healthy yeast, from a propagation if possible.
    - Consider a prop beer if you do not have a yeast propagator.
  - Old pitch suggestions of $1x10^6$/mL/°P do not apply! Our target is $3x10^6$/mL/°P, tank KO cell counts of $100x10^6$/mL!!
  - High aeration for a healthy Log phase. A target above 30ppm of O_2 is not crazy!

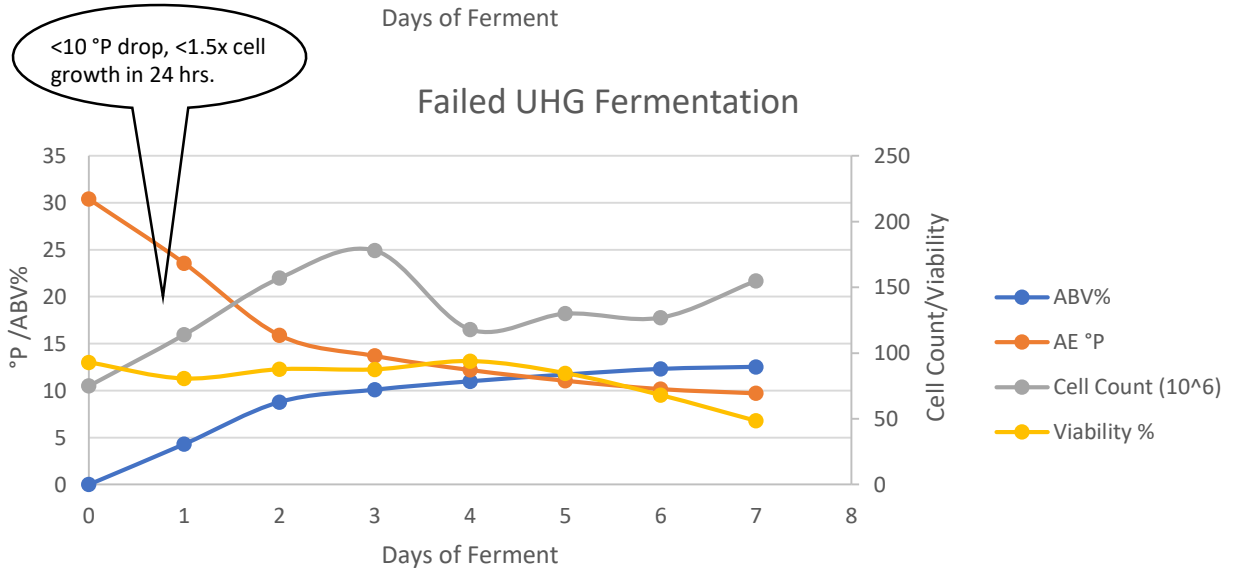
Fermentation

- You made it through the easy part of wort production and yeast management. Now how do you ferment this beast?
- Fermentation Temperatures
  - Lots of sugar = lots of metabolic heat = rapid fermentation and fusel alcohol production.
    - 18-20°C allows for good yeast growth over the first 24hrs. while reducing fusel production (stain dependent).
    - Vicinal diketone (VDK) rest must be respected. 5°C temperature raise at 50% AE is a good place to start for a productive VDK reduction.
- Yeast Cell Counts and Viability
  - CC's should peak around 48hrs., in our case $200x10^6$/mL is expected
  - CC's of >$175x10^6$ and viabilities >90% are expected within the first 48hrs.

Fermentation Curves “How to Predict the Unknown”

- Key Performance Indicators for UHG fermentations are very helpful
  - First 24hrs. KPI’s
    - >10°P drop in gravity
    - 1.5x cell growth off KO number
    - Viability =>90%
  - 72 hr. KPI’s
    - 50% AE, temp up for VDK rest
    - Around 2x cell count of initial KO number
    - Viability =>90%
  - Last 48hrs. KPI’s
    - °P is slowing to anticipated final AE
    - Viability begins to fall
    - %ABV near spec.





What Next?

- Autolysis is a concern with these beers.
  - Crash cooling and yeast removal ASAP!
  - Higher VDK's are "ok" in these beers, crashing <=150 ppm is not unheard of.
  - If you have a centrifuge, use it here for rapid yeast removal.
    - If you don't consider fining and scheduled yeast dumps on a daily basis.
- Barrel Aging
  - Now's the time to put this behemoth on oak if that's your plan.

High Gravity/Sour Brewing on a Small Scale

Jon Cross
Owner / Head Brewer
Call to Arms Brewing Co.

Berkeley Tart Blonde

5.0% ABV

Built for CO patio weather, this beer is pleasantly tart with notes of red-apple, pineapple and pear.

Add a caption.

High Gravity/Sour Brewing on a Small Scale

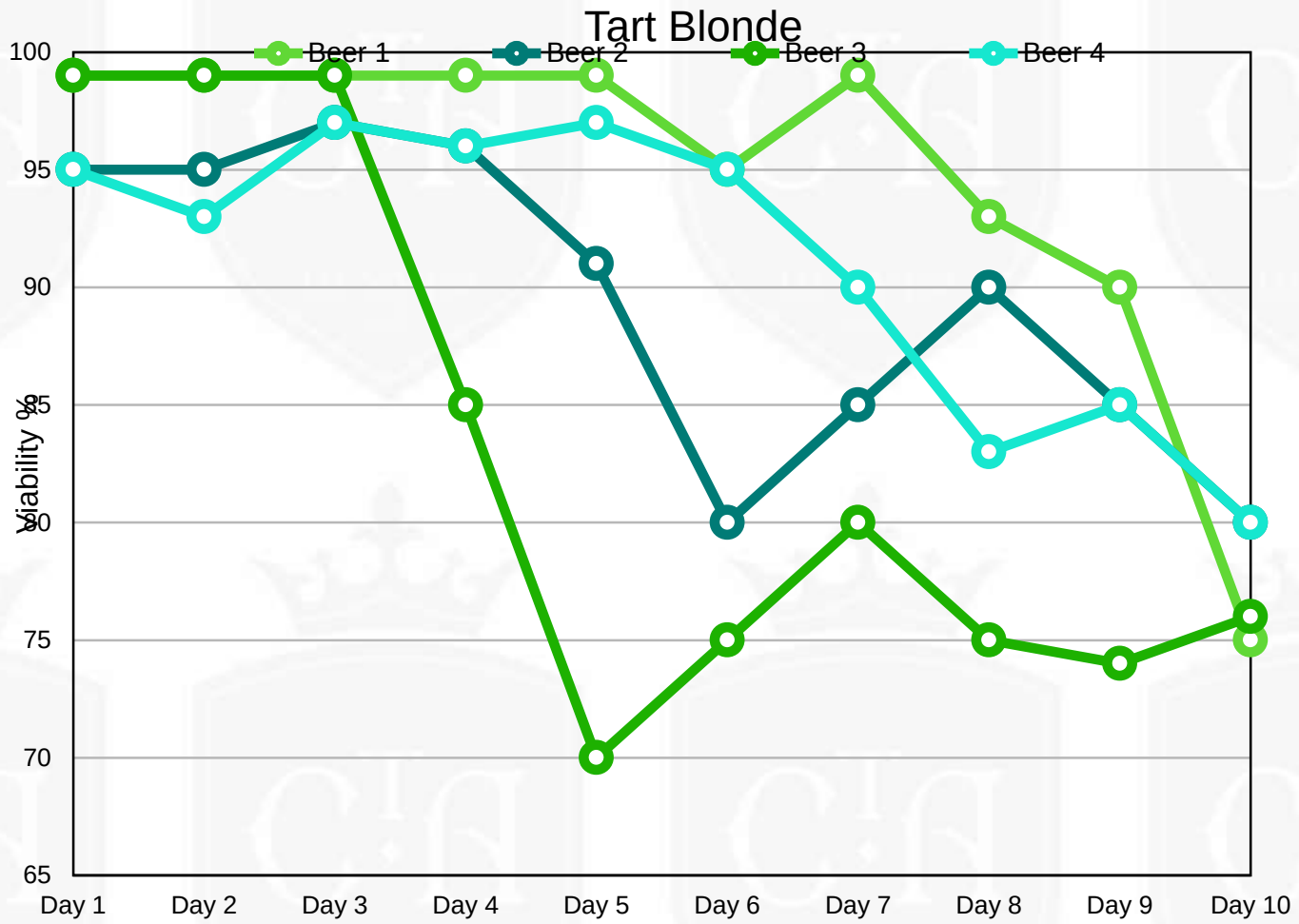
1. Healthy yeast (slurry viability >90%)
2. Huge pitch rate w/battle-tested strain
3. Daily lab work - cell counts and viabilities are a must
4. Sensory on the beer every single day
5. Small tanks are great. Sometimes less is more.

Yeast wrangling in a non-traditional process

- Bridging the gap between Old-World sours and a kettle sour
- No-hop 90 minute boil w/hot KO
- Give the bugs 24 hours to do work *(Lactobacillus delbrueckii)*
- Introduce healthy *Sacc.* to a stressful environment
- Carefully monitor yeast viability

- **What do you get?**
- Tons of ester production!
- Ethyl hexanoate, ethyl octanoate, etc.

Viability over time



Relatively quick drop in yeast viability compared to our standard fermentations

Where could this go wrong?

1. Yeast death/autolysis and associated off-flavors
2. Other microbial contamination
3. Re-pitching the yeast after fermentation
4. Stress + low reproduction = bad time

CROOKED STAVE®

ARTISAN BEER PROJECT

Technical Track I: "Yeast Health and Viability in High Gravity and Sour Beers"

L’Brett d’Or

Style – Méthode Traditionnelle Spontaneous

Spontaneously fermented in oak barrels, L’Brett d’Or’s fermentation is kicked off with the help of the natural wild yeast and bacteria native to our brewery. Since our spontaneous beers can only be brewed during the winter months, individual releases involve blending unique barrels to bring out the best of each brewing season. L’Brett d’Or will continue to age with the best of them, gaining complexity over the years.

Beer Specs

- Serving Temperature: 54°F
- Alc/Vol: 5.0%
- Cultured in Yeast

SOUR / FUNKY / RUSTIC / CRISP

Food Pairings:

- Jasper Hill Cellars Willoughby with washed-rind
- Saucisson Sec & Head Cheese
- Grilled Mushrooms Salad with Mustard Shallot Vinaigrette
- Grilled Clams with Herb Butter

Bottle Conditioning – Yeast Refermentation

Biology Basics

- Yeast metabolism in refermentation = EtOH and **CO2**

Bottle conditioned Beer

- "Finished Beer" + Sugar (Dextrose) + Yeast + Time (~2-6 weeks) + Temperature (~ 78°C)

CO2 Principals

- CO2 is dissolved in beer – lower temperature equals quicker CO2 absorption
- Most breweries measure in volumes of CO2
- Measuring devices – Anton Paar, Haffmans Gehaltemeter, Zahm

Current Methods of Conditioning

- Using high krausen wort
  - Problems with inaccurate cell counts and consistency
- Partial forced carb and bottle condition
  - Shorter time, better consistency, less complexity of flavor development
- Adding sugar and yeast to "finished" beer
  - Tradition, deep complex flavor development, can be very accurate, longer shelf life, somewhat reproducible

Current Method

- **Final Specs and Calculations**
  - ABV, FD, ADF & CO2 in still beer
  - Calculate yeast and sugars based on volume and CO2
- **Yeast**
  - DV10 Champagne yeast
  - 1.0×10^6 cells/mL
- **Nutrient**
  - Go Ferm
  - Comprised of essential micronutrients for yeast health
- **Hydration**
  - 30 mins
  - HLT water cooled to appropriate temperature
  - Temper with beer for 15 mins prior to pitch
- **Pitch and Package**
  - Pitch yeast and sugar, recirculate for 30 mins
  - Bottle ASAP, Lab gets 6 bottles, fully represent run
  - Storage

Complications of Bottle Conditioning

- Consistency
- Time and cost of holding product
- Temperature dependent
- Stalling or not conditioning due to high ABV and high levels of acid

What Now?

- Identify problem at Crooked Stave
- Beers >8% ABV and/or >15 g/L

Terminal Acidity Shock (TAS)

- Refers to the death or dormancy stage of yeast during fermentation or refermentation in high acidity and high ethanol containing beers. (Rogers et.al)

New Procedure?

New Procedure

- **Tempering**
  - Allow yeast to slowly adjust to extreme conditions using generation doubling increasing health and strength of yeast
- **Pseudo “YPD” media**
  - Use 2°P dextrose water with yeast nutrient to hydrate yeast, 48 hours prior to pitching
  - This allows ample time for yeast to hydrate and start consuming sugar
  - Incubate @ 28°C
- **Yeast monitoring**
  - Cell density and viability is checked after incubation
- **Beer addition**
  - After initial 24 hour period, add 50:50 blend of beer and 2°P sugar water
  - This allows for yeast to maintain viability while being introduced to future environment
  - Incubate @28°C
- **Pitch**
  - After 48 hours, yeast are ready for pitch, recirculation and bottling
  - Continue procedure as normal

Results

CO2 results

- More consistent carbonation in beers previously showing little to no conditioning
- 1.5x volumes of CO2 @ 2 weeks
- 2x volumes of CO2 @ 4 weeks
- Fully conditioned by 6 week mark

Yeast results

- First 24 hours of hydration
  - Yeast density held steady
  - Viability of rehydrated yeast also steady (95-99% viable)
- Next 24 hours (Beer addition)
  - Yeast density held steady
  - Viability of yeast slurry + beer (~95% viable)
  - Continued to monitor slurry for 5 days
  - Viability held steady until day 3, dropped to ~80%
  - Day 4 ~66%
  - Day 5 <50%