The Effect of Pitch Rate on Fermentation and Isoamyl Acetate Production

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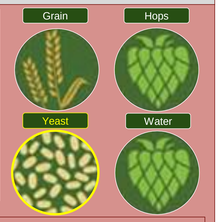
Introduction

- There are four main ingredients in beer: grain, hops, water, & yeast.
- Yeast is critical for beer fermentation. The yeast convert glucose into alcohol and CO_2, as well as many desirable esters.
- The amount of yeast added (pitch rate) is crucial for efficient fermentation and production of desirable flavors and aromas in the final product.

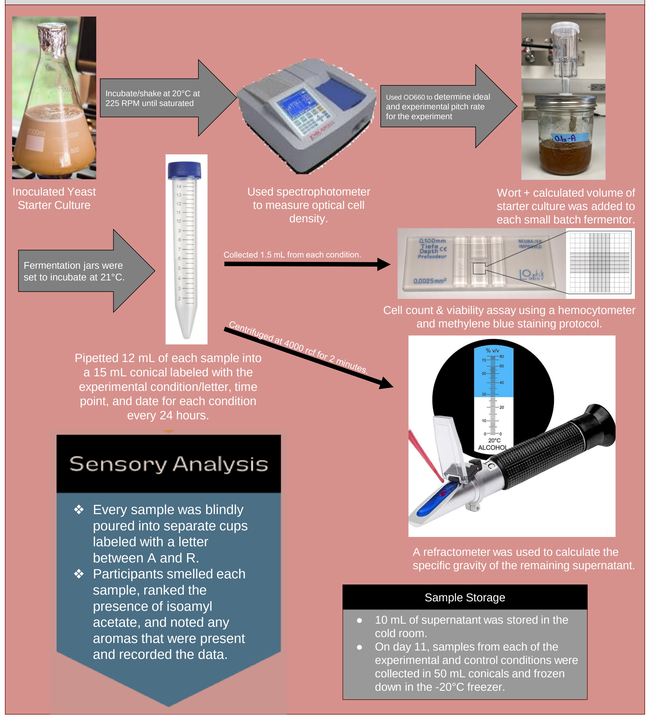


- Yeast strain suppliers and brewers claim that underpitching yeast for Hefeweizen strains increases isoamyl acetate production, a desirable aromatic compound for this beer style.
  - ➢ "Traditional brewing techniques suggest underpitching to produce more classic characteristics of the style" (White Labs).

Hypothesis

Yeast pitch rate will affect fermentation rates and isoamyl acetate production in a Hefeweizen brewing strain.

Materials & Methods



Sensory Analysis

- Every sample was blindly poured into separate cups labeled with a letter between A and R.
- Participants smelled each sample, ranked the presence of isoamyl acetate, and noted any aromas that were present and recorded the data.

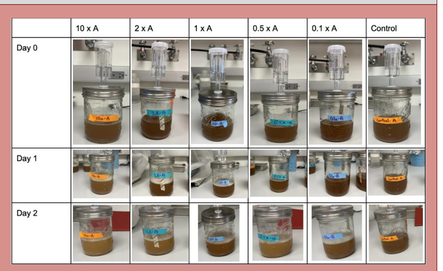
Sample Storage

- 10 mL of supernatant was stored in the cold room.
- On day 11, samples from each of the experimental and control conditions were collected in 50 mL conicals and frozen down in the -20°C freezer.

Results

Table 1: The Progress of Fermentation for All Conditions

- Based on the spectrophotometer OD660 value of 0.964, calculated values of yeast were added to each fermentation vessel to create the following conditions in triplicate:
  - ➢ 10-fold increase, 2-fold increase, ideal pitch rate, 2-fold decrease, and a 10-fold decrease.
- Fermentation is increasing between the day 0, day 1, and day 2 small batch fermentors.



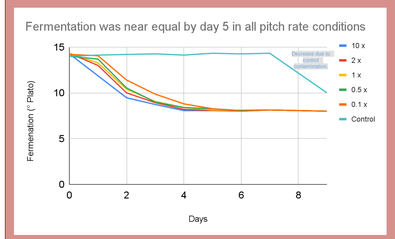


Figure 1: Average Fermentation Rates for All Pitch Rate Conditions.

- After day 5, fermentation rates dropped off in all conditions.

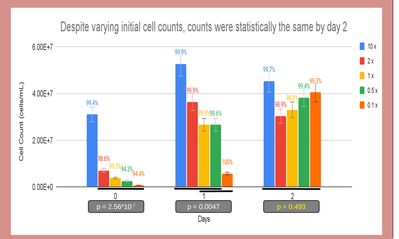


Figure 2: Average Cell Counts & Viability for All Pitch Rate Conditions.

- Cell counts were significantly the same by day 2 in all conditions.
- Viability was high at >94.3% for all conditions.

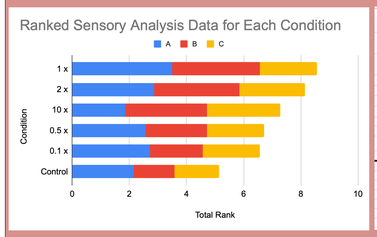


Figure 3: Ranked Sensory Analysis Data.

- On average, the ideal pitch rate had the highest observable isoamyl acetate levels.
- Overpitching was found to lead to higher detectable levels than underpitching.

	sum of squares	degrees of freedom	mean square	Fs	p	variance component (%)
among groups	0.611	5	0.122	2.285	0.067	15.51
within groups	1.925	36	0.053			84.49
total	2.536	41				

	10x	2x	1x	0.5x	0.1x	control
mean	0.765714	0.867143	0.885714	0.722857	0.712857	0.52
n	7	7	7	7	7	7

group names →	10x	2x	1x	0.5x	0.1x	control
data→	0.83	0.83	0.88	0.33	1	0.5
	0.92	1	0.92	0.92	0.75	0.75
	1	0.44	1	0.78	0.89	0.67
	0.8	1	0.9	1	0.8	0.6
	0.58	1	0.83	0.33	0.42	0.42
	0.9	0.8	1	0.7	0.8	0.7
	0.33	1	0.67	1	0.33	0

Figure 4: One-Way ANOVA with Standardized Isoamyl Acetate Scores.

- The p-value was 0.067 which is >0.05 and is not statistically significant.

Further Directions

- Scale the experiment up to better imitate brewery conditions to account for the control of oxygen levels seen in brewery fermentation tanks.
- Incrementally underpitch the beer to see if there is a condition that is statistically different that was not tested in this experiment.
- Work with a trained cicerone for sensory analysis who can better quantify the data.
- Perform GC-MS on samples to quantify isoamyl acetate levels.
- Examine gene expression in all conditions and determine correlation with isoamyl acetate production.

Discussion

- Fermentation rates, as expected, were higher in over pitched conditions at the start, but by the five day mark attenuation in all pitch rate conditions was equal.
- Cell counts in all pitch rate conditions equilibrated by day 2 regardless of pitch rate.
- Control contamination is evident indicated by visual identification of cells on the refractometer and decreased specific gravity which signifies increased fermentation.
- The sensory analysis of control B revealed a diacetyl aroma, which could be indicative of contamination with lactic acid fermenting bacteria. Controls A & C had aromatics of other fruity esters, but the strong presence of isoamyl acetate was not observed.
- On average, the presence of isoamyl acetate was observed, but not as a strong characteristic. Other esters were also observed such as pear, apple, and clove-like aromas.

Conclusion

- While pitch rate affected the initiation of fermentation, it did not have a significant impact on fermentation length or attenuation.
- There was no significant difference in cell count or viability by day 2 of the experiment.
- This experiment aimed to find the specific underpitch condition that will produce significant amounts of isoamyl acetate.
  - ➢ While there was no statistical difference in the sensory data, the average data trends revealed that the isoamyl acetate levels were highest at ideal pitch rate.

Citations

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