Examining the Role of *ATF1*, *ATF2* & *IAH1* in the Production of the Banana Aromatic, Isoamyl Acetate, During Fermentation in Brewing Yeast

Jenna Vesey and Joost Monen
Ramapo College of New Jersey, Mahwah, NJ, 07430

Introduction

Beer has brought communities together for generations

- Beer dates as far back as ancient mesopotamia and egypt, found in ancient art and tombs.
- Gave rise to the American Saloon.
  - ➢ Social life of 1800s.

The brewing process has remained largely unchanged

- There are 4 main ingredients in all beers:

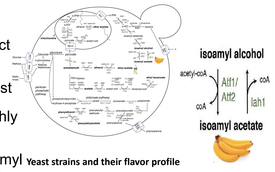
- The craft of brewing, while still remaining much the same, has become much more methodical as we learn about the science behind the process.

Yeast plays a major role in the production of aromatic compounds giving beer its flavor

- Beer styles from around the world have distinct flavor and aroma characteristics.
- Yeast not only produce alcohol, buy play a major role in the production of flavor compounds during the fermentation process.

glucose —yeast→ ethanol + carbon dioxide

- Selective pressure to produce beer with distinct characteristics has led to the evolution of a variety of yeast strains.
- Brewers can now select from hundreds of yeast strains to impart flavor to their beer.
- Isoamyl Acetate is a banana aroma that is highly desirable in certain beer types, particularly Hefeweizen.
- 3 genes involved in the the conversion of isoamyl alcohol (very weak aromatic flavor) to isoamyl acetate include *ATF1* and *ATF2* (Alcohol Acetyl Transferase) and *IAH1* (Isoamyl acetate-hydrolyzing esterase).
- How these genes are expressed to produce differing levels of isoamyl acetate between strains is poorly understood.



Yeast strains and their flavor profile

Strain	Flavor Profile
WLP090 - San Diego Super Ale	Low Ester Producing/ Neutral
WLP001 - California Ale	Clean/Neutral
WLP565 - Belgian Saison Ale	Earthy/Spicy/Fruity
WLP300- Hefeweizen Ale	Banana and Clove

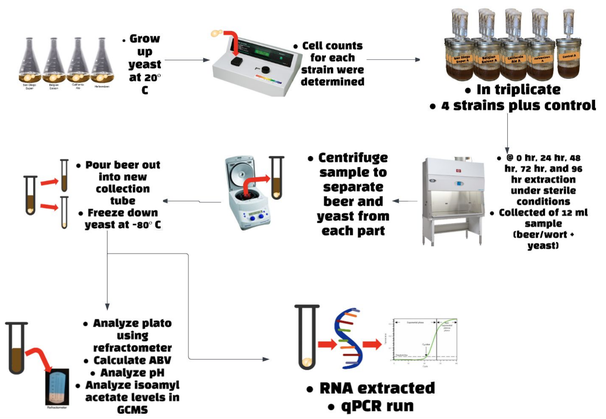
QUESTION

What is the relationship between gene expression of *ATF1*, *ATF2*, and *IAH1* and isoamyl acetate production?

HYPOTHESIS

Strains producing higher levels of isoamyl acetate will have higher gene expression of *ATF1* and *ATF2* and lower levels of *IAH1* expression, driving the reaction forward.

Materials and Methods



Results

Beer fermentation rate is largely unaffected by strain selection

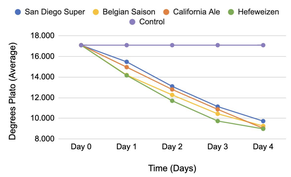


Figure 1: Wort/Beer density monitored at 24 hr timepoints.

- Specific gravity measurements in degrees Plato (a measure of density of wort/beer, which indicates the degree of fermentation taking place) were taken at 24 hr timepoints.
- There was no significant difference between timepoints, except at the 72 hr timepoint (one-way ANOVA: p = 0.0034)
  - ➢ Tukey-Kramer test revealed Hefeweizen was significantly different from California Ale and San Diego Super.

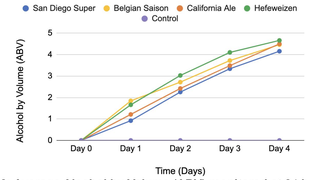


Figure 2: Average Alcohol by Volume (ABV) monitored at 24 hr timepoints.

- To calculate ABV, plato was converted to specific gravity using the equation: SG = 1+ (plato / (258.6 – ((plato/258.2) *227.1)))
- ABV was then calculated from this specific gravity using the following conversion: ABV = (gravity day 0 - gravity day X) * 131.25
  - ➢ Where X is the specific day

Belgian Saison has the highest gene expression in all three genes

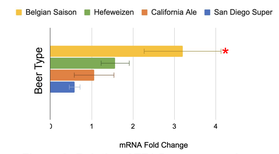


Figure 3: Relative *ATF1* Gene expression

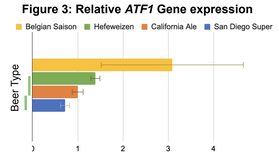


Figure 4: Relative *ATF2* Gene expression

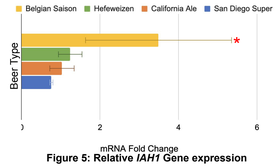


Figure 5: Relative *IAH1* Gene expression

- mRNA expression fold calculated using $\Delta\Delta$Cq method.
- *ACT1* was used as a reference gene compared to California ale as the control strain.
- **Figure 3**: There was significant difference between Belgian Saison and every other type of beer (one-way ANOVA: p = 0.0022).
  - ➢ Tukey-Kramer test revealed that the Belgian Saison was significantly different from every other beer type.
- **Figure 4**: There was significant difference between beer types (one-way ANOVA: p = 0.026).
  - ➢ Tukey-Kramer test revealed San Diego Super was significantly different from Belgian Saison.
- **Figure 5**: There was significant difference between Belgian Saison and every other type of beer (one-way ANOVA: p = 0.028).
  - ➢ Tukey-Kramer test revealed that the Belgian Saison was significantly different from every other beer type.

Hefeweizen has the highest levels of isoamyl acetate

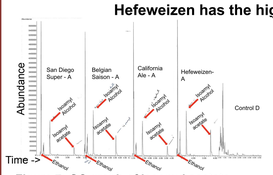


Figure 7: GC graph of isoamyl acetate levels

- GC data comparing peaks of ethanol, isoamyl alcohol, and isoamyl acetate.
- Hefeweizen has the highest levels of isoamyl acetate.

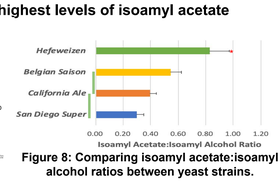


Figure 8: Comparing isoamyl acetate:isoamyl alcohol ratios between yeast strains.

- The Hefeweizen strain has a significantly higher isoamyl acetate to isoamyl alcohol ratio (one-way ANOVA: p = 0.000825).
  - ➢ Green bars indicate no significance between strains, and asterisk indicates significant difference (Tukey-Kramer test).

The ratio of gene expression of *ATF1&2* and *IAH1* is important in isoamyl acetate production

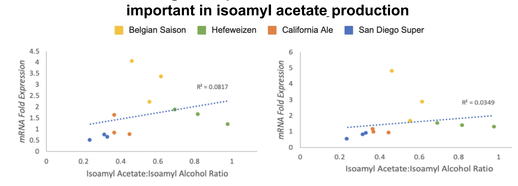


Figure 9: ATF1 mRNA fold expression vs Isoamyl Acetate:Isoamyl Alcohol

Figure 10: ATF2 mRNA fold expression vs Isoamyl Acetate:Isoamyl Alcohol

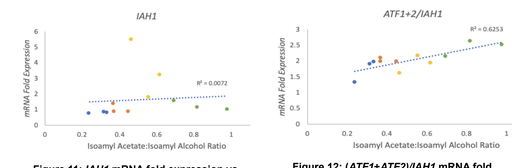


Figure 11: *IAH1* mRNA fold expression vs Isoamyl Acetate:Isoamyl Alcohol

Figure 12: (*ATF1+ATF2)/IAH1* mRNA fold expression vs Isoamyl Acetate:Isoamyl Alcohol

- The figures above represent XY scatter plots comparing mRNA fold expression to isoamyl acetate:isoamyl alcohol ratio.
- There is not a strong linear correlation when plotting any individual gene's expression vs. isoamyl acetate:isoamyl alcohol ratio.
  - ➢ When Belgian Saison data is removed these correlations become much more pronounced (data not shown).
- When factoring in the balance of *ATF1* & *AFT2* driving the reaction forward, and *IAH1* driving the reverse reaction, there is a strong linear correlation (r = 0.791) when comparing (*ATF1+2*):*IAH1* ratio vs. isoamyl acetate:isoamyl alcohol ratio.

Discussion

- All of the strains examined show comparable fermentation rates, with no significant difference in alcohol by volume after 96 hours of fermentation.
- Isoamyl acetate production differs between strains, with the Hefeweizen producing the highest levels.
- Gene expression for all 3 genes was significantly higher in Belgian Saison then in any other strain tested.
- There was not an obvious correlation between any single gene examined and isoamyl acetate production indicating that there is not one single gene controlling isoamyl acetate levels.
- Isoamyl acetate production appears to be driven by the balance of ATF1, ATF2, and IAH1 expression, with higher expression of *ATF1&2* and lower expression of *IAH1* leading to more isoamyl acetate levels.

Future Directions

- Look at various time-points throughout fermentation to examine how gene expression changes, and how isoamyl acetate levels change.
- Examine more yeast to see if the correlation between gene expression and isoamyl acetate production is consistent from strain to strain.
- Examine gene expression and isoamyl acetate production in a brewery setting to corroborate results seen in the lab.
- Genetically modify yeast to upregulate *ATF1 & ATF2* and/or downregulate *IAH1*.

Acknowledgement

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