Petite Mutations and their Impact of Beer Flavours

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Table of Contents

- Introduction
- Experimental Design
- Results
- Discussion
- Conclusion
What Are They?

- No or reduced mitochondrial function
- Spontaneous genetic mutation
- Also called respiratory deficient (or RD) cells

Figure 1. TTC Overlay Technique with Petite Mutations Noted with an Arrow
Petites in the Industry

- Exist in frequencies of:
  - 0.1% to 0.5%
  - 1% to 4%
  - Strain Dependent
- Higher if yeast is abused
“The RD mutant produced considerably higher amounts of isobutanol and isoamyl alcohol”

Ernandes, 1993
Method for Fermentation (ASBC Yeast-14)

Introduction

Experimental Design

Results

Discussion

Conclusion

Ethidium bromide to produce 3.66, 5.10, 8.67, and 10.77% RD's measured by TTC Overlay method
Mini-fermentation Assay

- Originated for flocculation determination
- Use 12.6 °P wort plus 4% glucose & 3 reps,
- Turbidity/density measurements taken 10 x’s @ 21°C over 75 h,
- Model sugar consumption and yeast in suspension,
- Standard method ASBC Yeast-14
Mini-fermentation Assay

Key to assay is the analysis of both change in °P/SG and \( A_{600} \).

These non-linear curves can be fit with Excel and statistically compared with Prism™.

\[
F(t) = Rt + Ae^{-\frac{(t-\mu)^2}{\sigma^2}}
\]

\( A \) – Amplitude
\( \mu \) – Mean
\( \sigma \) – Standard Deviation
\( R \) – Rotation (slope term)

Height of peak, \( P = Rt + A \)
Figure 2. Yeast in suspension trends with fermentations containing varying levels of petites.

No significant (p>0.05) difference for yeast in suspension characteristics as the petite mutations present in the fermentation increased.

Introduction
Experimental Design
Results
Discussion
Conclusion
Method for Fermentation (ASBC Yeast-14)
**Flavours Analyzed**

- “Significant” means the flavour compounds present in the beer changed as the percentage of petites changed.
- No significant difference in all higher alcohols analysed and acetone.

**Table 1. Results showing if there was a significant change in flavour compound levels post beer fermentation as the percentage of petites present increased up to 11%**

<table>
<thead>
<tr>
<th>Flavour Compound</th>
<th>Significant (p&gt;0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaldehyde</td>
<td>Yes</td>
</tr>
<tr>
<td>Butanedione</td>
<td>Yes</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>Yes</td>
</tr>
<tr>
<td>Isobutyl acetate</td>
<td>Yes</td>
</tr>
<tr>
<td>Ethyl butyrate</td>
<td>Yes</td>
</tr>
<tr>
<td>Pentanedione</td>
<td>Yes</td>
</tr>
<tr>
<td>Ethyl Octanoate</td>
<td>Yes</td>
</tr>
<tr>
<td>Iso amyl acetate</td>
<td>Yes</td>
</tr>
<tr>
<td>Ethyl hexanoate</td>
<td>Yes</td>
</tr>
<tr>
<td>3-Methyl butanol</td>
<td>No</td>
</tr>
<tr>
<td>2-Methyl butanol</td>
<td>No</td>
</tr>
<tr>
<td>Propan-1-ol</td>
<td>No</td>
</tr>
<tr>
<td>Isobutanol</td>
<td>No</td>
</tr>
<tr>
<td>Acetone</td>
<td>No</td>
</tr>
</tbody>
</table>
Acetaldehyde Levels Post Fermentation

- Linear increase in acetaldehyde level post fermentation with increasing levels of petite mutations
- Same trends found with other eight flavour compounds

Figure 4. Acetaldehyde levels in the beer post fermentation as the percentage of mutated cells increased in the population

$R^2 = 0.9431$
$p > 0.0001$
Flavours Analysed

- Flavours outlined showed a significant change in flavour levels as the petite mutations in the pitching yeast changed.

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Introduction  | Experimental Design  | Results  | Discussion  | Conclusion
Does increase in flavour compounds make noticeable differences?

**Acetaldehyde**
- Flavour Threshold – 5mg/L
- From experimental values, a 10% increase in petites would result in a 1.44mg/L increase in acetaldehyde

**Isoamyl acetate**
- Flavour Threshold – 1.1mg/L
- 10% increase in petites would result in 0.58mg/L increase in isoamyl acetate

Introduction
Experimental Design
Results
Discussion
Conclusion
Does increase in flavour compounds make noticeable differences?

**Ethyl Acetate**
- Flavour threshold - 5mg/L
- With 10% increase in petites, 5.6 mg/L increase of ethyl acetate

**Butanedione**
- Flavour threshold – 0.01mg/L
- 10% increase resulted in 0.05mg/L increase in butanedione

**Possible Index Compounds?**

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Introduction  | Experimental Design  | Results  | Discussion  | Conclusion
Conclusions from Petite Mutation Experiment

- At low levels of petites, flocculation characteristics didn’t change.

- Nine of the flavour compounds analysed increased with increasing petite mutations in the population.

<table>
<thead>
<tr>
<th>Ethyl octanoate (mg/L)</th>
<th>% Petite Mutations</th>
</tr>
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<tbody>
<tr>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>0.1</td>
<td>5.0</td>
</tr>
<tr>
<td>0.2</td>
<td>10.0</td>
</tr>
<tr>
<td>0.3</td>
<td>15.0</td>
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</tbody>
</table>

Graph showing absorbance at different time points for different petite mutation percentages.

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Introduction

Experimental Design

Results

Discussion

Conclusion
Conclusions continued

- Higher alcohols or acetone did not change with increasing levels of petites
- Based on results, it’s likely ethyl acetate and butanedione will be the first noticeable flavour changes in beer if a high number of petites are present
Thank you for listening,

Questions?

Acknowledgements

IBD Ph.D. Studentship – M Josey

Alltech ICBD donation
References

Increasing levels of petite mutations showed no significant (p>0.05) in propan-1-ol.

Same trends found with other higher alcohols and acetone.

Figure 3. Propan-1-ol (mg/L) in beer fermented with varying levels of petite mutated yeasts.
Flavour compounds present in beer increase as the percentage of mutated cells increase.

- **Iso amyl acetate (mg/L)**
  - $R^2 = 0.844$
  - $p > 0.0001$

- **Ethyl hexanoate (mg/L)**
  - $R^2 = 0.8291$
  - $p > 0.0001$

- **Ethyl octanoate (mg/L)**
  - $R^2 = 0.9449$
  - $p > 0.0001$
“The Ehrlich pathway and the main genes involved in the synthesis of enzymes catalyzing each reaction. The reversible transamination reaction uses different BAT-encrypted enzymes—while BAT2 catalyses the transfer of the amino group from the amino acid to α-ketoglutarate (AKG), BAT1 is usually required on the reverse transamination for amino acid biosynthesis” (Pires, Teixeira et al. 2014)
"A scheme of the chemical reactions involving the biosynthesis of acetate esters (a) and medium-chain fatty acid ethyl esters (b). The main genes involved in each reaction are presented above the reaction arrows" (Pires, Teixeira et al. 2014)