Wort & Yeast Nutrition Effects on Flavour

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This topic is a challenge for all.

And certainly old professors!
Outline

- Introduction
- Flavours
- Wort Nutrients
  - Oxygen
  - Lipids
  - Sugars
  - Nitrogen/FAN
  - Zinc/Minerals
  - Esters
  - Example of ester interactions
  - Higher OH’s
  - DMS
  - Acetaldehyde
  - VDK’s
  - 4VG
- Hops (really)
- Acknowledgements
Introduction

- While carbohydrates and yeast produce alcohol
  - CHO $\rightarrow$ Ethanol

- The process is much more complicated than that!
  - Biochemistry
  - Materials
  - Process
Introduction

Fig. 2. Overview of the most common targets for enhancing the performance of brewing yeast (↑ - increase, ↓ - decrease, ↑↓ - in equilibrium).
Introduction

Yikes!
Introduction

- This talk will focus on how factors that brewers can alter:
  - Wort composition/ $O_2$
  - Yeast strain/pitch rate/timing
  - Fermenter temperature profile
  - Tank type/height??

- Modulate nutrient’s effect on flavours.
<table>
<thead>
<tr>
<th>Fermentation Product</th>
<th>Levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol, CO$_2$, Glycerol</td>
<td>g/L</td>
</tr>
<tr>
<td>Higher OH's, Esters, Organic acids</td>
<td></td>
</tr>
<tr>
<td>Short chain fatty acids</td>
<td></td>
</tr>
<tr>
<td>Aldehydes, SO$_2$, Keto acids</td>
<td></td>
</tr>
<tr>
<td>Acetoin, 2,3 Butanediol, Diacetyl</td>
<td>mg/L</td>
</tr>
<tr>
<td>H$_2$S, DMS, 4VG</td>
<td>µg/L</td>
</tr>
</tbody>
</table>
Oxygen

- Brewers control yeast growth and metabolism. By controlling $O_2$ More $O_2$ more yeast, less ethanol.

- Yeasts can be divided into classes:
  - 01—yeasts whose requirement is satisfied if wort is half saturated with air;
  - 02—yeasts whose requirement is satisfied by air saturated wort;
  - 03—yeasts whose requirement is satisfied by oxygen-saturated wort;
  - 04—yeasts whose need is not satisfied by oxygen-saturated wort.

- Often 1mg/L per °P used as a guide.
Making Sense

- Very variable
- Pitching hemocytometer error ~10%
Oxygen

- From Casey’s fishbones:
  
a) @ 5-15% used for sterol synthesis,
b) @ 15% used for UFA synthesis,
c) @ 70% consumed in oxidation of wort constituents.

- Standardize your sampling point/time to check DO and control process target DO around this.
Sorry I had to mention it again! First in 2006,

Again in 2010 &,

Again in 2013,

So let's just say it can have a minimal or maximal effects on flavour effect
Lipids

- Wort lipids (in trub) can partially substitute for $O_2$

- However, much of trub’s action is due to trub particles acting as nucleation sites for $CO_2$ thus mitigating $CO_2$‘s toxic effect on yeast.

- Trub also binds Zinc
Sugar

- While wort sugars are often considered simply as energy and a route to ethanol production the type of sugar does influence beer flavour.

- For example Stewart and coworkers demonstrated that the substitution of maltose syrups for ‘malt’ sugars can decrease the levels of esters and higher alcohols in HG worts. Use to flavour match normal vs HG and VHG worts.
Sugar uptake

- Maltose
  - permease
  - $\alpha$-glucosidase
- Maltotriose
  - permease
  - $\alpha$-glucosidase
- GLUCOSE
  - transporter
- Maltose
- Maltotriose
  - GLUCOSE
- Fructose
- Glucose
  - Glucoamylase
  - Starch/Dextrin
- Glucose + Fructose
  - Invertase
  - Sucrose
Sugar uptake
Nitrogen

- Normally one needs greater than ~230 mg/L of Free or Fermentable Amino Nitrogen (FAN),
- High FAN high yeast growth, high diacetyl,
- Low FAN slow fermentations and high diacetyl,
Nitrogen

Extended boil increases browning and decreases FAN both resulting in slower and lower fermentations.
Zinc

- Can be deficient in wort with high adjuncts,
- Need 0.08 mg/L up to 0.30 mg/L (essential for yeast)
- As levels to 0.30 mg/L fermentation increases,
- Absorbed by trub. If trub present in the fermentation yeast may be use Zn based in trub.
- Add as ZnCl$_2$ or ZnSO$_4$ or as ‘Servomyces’ yeast as close to fermenter start as possible.
Magnesium and Calcium

- Mg and Ca are essential for a multitude of enzymatic activities,
- Mg needed 42.5-25000 mg/L,
- Ca needed 20-1000 mg/L,
- An increased ratio of Mg to Ca causes:
  - an increase in the initial fermentation rate,
  - rate and yield of ethanol produced and
  - an increase in vitality at the end of fermentation
- it appears there is little direct flavour impact but for possible oxidation by Mg
- Ca at high levels imparts an astringency
Ester Formation

Levels influenced by:
- Barley & Malt *
- Yeast Strain *
- Wort Aeration -
- Wort sugar spectrum *
- FAN levels + high or low
- Wort lipid/turbidity -
- Fermentation
- Temperature +
- Pitch Rate -
- OG +
- Fermenter height -
Ester Formation

PROCESS CONTROL FOR ESTERS IN BEER: NUTRITIONAL CONSIDERATIONS

OXYGEN & TRUB

- Under aeration (+)
- Sterols (-)
- Extremely low D.O., e.g., < 1 ppm (-)
- Increase wort lipids...decrease acetate esters (-)
- Wort D.O.'s between 1.5-6 ppm (+)
- High trub, high lipid worts...more uptake of linoleic by yeast...will affect esterase activity...less esters made.
- Scottish Courage wort OG is the most influential parameter on (ethyl acetate)...direct correlation between fermentation temps of 16-20°C
- Acidified sparge water...leads to less astringent materials in beer, allowing for beer ester character to "come through" e.g., sparge at pH 5.7 vs. 6.0.
- Suntry: Happoshu beers generally lower in esters, especially EA and IAA.

High unsaturated fatty acids (+) (especially linoleic)

"Preoxygenation" of slurry yeast...oxygenate yeast directly 2-4 hours before pitching to reach a reduced oxygen content, not through wort leads to:

a) [sterols] increases 1.5-4.0 fold
b) [UFAs] increases 1.5-4.0 fold
c) 25% increase in acetate esters (88% for IAA alone!)
d) 21% increase in higher alcohols...strain dependent magnitude.

FAN & ZINC

- With excess FAN & D.O., high yeast growth yields proportionally more esters due to higher [ of cells (+)
- Scottish Courage, studies over 1955-1965 OG worts, syrup at 0-10%; zinc at 9.2 vs. 0.8 ppm, D.O. at 8 vs. 20 ppm:
  a) Ethyl acetate: OG (+), syrup (+)
  b) Phenyacetate: OG (+), zinc (+) and syrup (+)
  c) Ethyl hexanoate: OG (+), syrup (+)
  d) Ethyl acetate: OG, zinc & syrup all (+)
- ASBC No. XIllb: (Casey; June/05)
- As an initial stage had little effect.
- Zinc (+), acts as a co-factor in reaction supplying alcohol: High FAN and low lipids (+)
- Low FAN & high lipid (-)
- Increase wort zinc...increase acetate esters.
- Increase wort FAN...little effect on acetate esters.
- Hep variety "Nelson Sauvin" from New Zealand...impacts a "grape-like flavor" to beer!

GGS: Overall, clear worts result in the production of less volatile acetate esters compared to cloudy worts.
- Wort gravity (+, disproportionate to lower gravity ratio of ethyl acetate and IAA esters.
- GGS: adding CaCl₂ to clear wort with DE present will decrease levels of acetate esters in beer even lower than levels from beers with cloudy wort and no DE addition...adding CaCl₂ is (+)

HOB: 
- Disproportionate production of ethyl acetate and IAA esters.
- "Dried Fruit" flavor by GC-OLF linked to beta-damascenone.

Wort OG & acetate esters (+)
- Low enzyme or unmodified malt (+)
- Percent maltose (-)
- Sulfhydryl reagents (-)
- HOB (+, especially ester acids (reduces ethyl hexanoate).

Bill of Materials

Scottish Courage: OG worts, syrup at 0-10%; zinc at 9.2 vs. 0.8 ppm, D.O. at 8 vs. 20 ppm:
- Ethyl acetate: OG (+), syrup (+)
- Phenyacetate: OG (+), zinc (+) and syrup (+)
- Ethyl hexanoate: OG (+), syrup (+)
- Ethyl acetate: OG, zinc & syrup all (+)
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Bill of Materials
Esters

The chemical formulas of organic esters usually take the form $\text{RCO}_2\text{R'}$, where $\text{R}$ and $\text{R'}$ are the hydrocarbon parts of the carboxylic acid and the alcohol, respectively.

e.g. ethyl acetate, iso-amyl acetate etc.
Effect of Temperature and Oxygen on Higher Alcohols/Esters

<table>
<thead>
<tr>
<th>Temperature</th>
<th>11°C</th>
<th>18°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>O₂ level mg/L</td>
<td>5</td>
<td>20</td>
</tr>
<tr>
<td>Esters</td>
<td>39</td>
<td>27.5</td>
</tr>
<tr>
<td>Higher OH’s</td>
<td>174</td>
<td>215</td>
</tr>
</tbody>
</table>
Higher Alcohol Formation

Levels influenced by:

- Yeast Strain **
- Fermentation Temperature +
- Pitch Rate -
- OG +
- Wort Aeration +
- FAN levels +
  \( (<15 \text{ or } >0.22 \text{ mg/L}) \)
- Zinc*
- Fermenter height +
DMS

- Cream Corn Aroma
- Green beer???
- Level influenced by:
  - Malt
  - Kettle Boil – time/extent
  - Whirlpool/ Hot Wort timing
  - Fermenter CO$_2$ ‘collection’
- Rarely due to bacterial contamination.
Acetaldehyde

- Green apple aroma
- Green beer???

Level influenced by:
- Yeast Strain **
- FAN (low) –
- Fermentation Temperature +
- Pitch Rate +
- Zinc –
- Oxygen –
- Pressure +

Viable yeast in maturation normally reduces levels below the taste threshold.
Diacetyl

- diacetyl
- maltose
- \( \alpha \)-acetolactate

\[ \text{CHO metabolism} \]

\[ \begin{align*}
\text{2 NADH} & \rightarrow \text{2 NAD}^+ \\
\text{O} & \text{O} \\
\text{\( \alpha \)-acetolactate} & \rightarrow \text{valine}
\end{align*} \]

\[ \text{CH}_3\text{C C CH}_3 \]

\[ \text{CO}_2 + [\text{2H}] \rightarrow \text{diacetyl} \]

\[ \text{2,3 butane 3 diol} \]
Diacetyl Diagnostics

- from L Kruger
- Method:
  - Sample beer from the fermenter
  - Remove the yeast (filter through filter paper)
  - Split in two:
    - A
      - 1/2
      - Refrigerate 1/2 hr
      - Taste
    - B
      - 1/2
      - Heat to 60°C for 1/2 hr
      - Cool and taste
Diacetyl Diagnostics

- **Interpretation I**
  - A tastes fine  B tastes of diacetyl
  - Precursor left in beer that will go to diacetyl over time (accelerated with heat)

- **Interpretation II**
  - A tastes of diacetyl  B tastes of diacetyl
  - (Same intensity)
  - Diacetyl left end of fermentation
  - Interpretation 3)

- **Interpretation III**
  - A tastes of diacetyl  B tastes of diacetyl
  - (B more intense than A)
  - Diacetyl and precursor left at end of fermentation
4-vinyl guaiacol (4VG)

- 4VG common in ‘Wheat’ beers
- Generated by POF gene which modifies ferulic acid to 4VG
- Clove-like aroma

Mash to ferulic acid

Yeast

4VG
Hops

Well….. dry hopping??

O₂???
R.E.2%↓
OH 1%↑
Closing points

● Each one of these flavours could be a talk in itself – the presentation is a summary!

● I doubt anyone knows all of this knowledge in detail.

● Remember Practice beats theory

● But speak up if you do!

● <Alex.Speers@Dal.ca>
Acknowledgments

- MBAA & Ontario Section
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And the Canadian Taxpayers!
Questions?