When Beer Goes Sour: An NMR Investigation

ADAM DICAPRIO
PROCESS NMR ASSOCIATES
87A SAND PIT ROAD DANBURY, CT 06810
What is Sour Beer?

- Wikipedia says:
  - “Sour beer is a beer style characterized by an intentionally acidic, tart, sour taste.”

- “Wild Brews: Beer beyond the Influence of Brewer’s Yeast”

- Category 17 of the Beer Judge Certification Program
  - Encompasses: Berliner Weisse, Flanders Red Ale, Flanders Brown Ale, Lambic, Fruit Lambic, Gueuze
What goes in to a sour beer?

- **Grain**
  - Malted Barley, Unmalted Wheat, Specialty Malts
    - Dextrins, Dextrins, Dextrins
  - *Sikaru* beer (3000 B.C.) – 62.5% Barley Malt + 37.5% Raw Wheat
  - Modern Lambic – Brasserie Cantillon recipe – 65% Barley Malt + 35% Raw Wheat

- **Hops**
  - Aged & Oxidized

- **Aging Vessels** – A sour beers home for up to a full century
  - Oak Barrels (French & American)
  - Oak Foudre
  - Stainless Steel Tank

http://www.belgianbeermagazine.com/oud-beermel-brewery/
Who goes in to a sour beer?

- Dozens of organisms\textsuperscript{2,3}
  - Bacteria
    - \textit{Enterobacteriaceae}
      - \textit{Citrobacter spp., Enterobacter spp., Klebsiella spp., Hafnia spp.}
    - \textit{Lactobacillaceae}
      - \textit{Pediococcus spp., Lactobacillus spp.}
    - \textit{Acetobacter spp.}
    - \textit{Klebsiella spp.}
  - Yeasts
    - \textit{Kloeckera apiculata}
    - \textit{Saccharomyces spp.}
    - \textit{Brettanomyces spp.}
    - \textit{Pichia spp.}
    - \textit{Candida spp.}
    - \textit{Hansenula spp.}
    - \textit{Cryptococcus spp.}
Why is Sour Beer Sour?

- Straight Lambic, Flanders Ales, Gueuze, Berliner Weisse
  - Lactic, Acetic, Succinic Acid
    - 85% - 10% - 5%
- Fruit Lambics
  - Cherries, Grapes – Malic Acid
  - Raspberries – Citric Acid
Chemistry of Sour Beers

- Application of Quantitative NMR to Biologically Acidified Mashes

- Quantitative NMR and Descriptive Chemistry of American Wild Ales and genuine Belgian Lambic
Berliner Weisse & Biological Acidification

- Reinheitsgebot of 1516
  - Beer can contain only malt, hops & water
    - Unmalted wheat and yeast added in the Provisional Law of 1996
  - Artificial alteration of pH is illegal
    - Development of Biological Acidification/Sour Mashing
      - Utilization of native microbes for pH adjustment

- Perform Starch Conversion
- Cool to ~120°F
- Innoculate
- Hold Temperature (120°F)
Temperature Dependence of the Sour Mash Technique

Goal:

- Does the “magic” temperature of 120°F have a chemical significance?
- Record and quantify sour metabolites & contaminant products as a function of sour mash temperature
  - Determine wt% of metabolites using Maleic Acid internal standard & manual integration
    - Lactic Acid
    - Acetic Acid (contaminant)
    - Succinic Acid
    - Ethanol
    - \(\gamma\)-Amino Butyric Acid (contaminant)
Straight Run of 95°F Sour mash; Assignments from Rodrigues et al. 2010
Freeze Dried Run of 95°F sour Mash; Assignments from Rodrigues et al. 2010
Absolute Metabolite Proportions

Metabolite Weight % vs. Temperature

- Lactic Acid
- Acetic Acid
- Succinic Acid
- Poly. (Lactic Acid)
- Poly. (Acetic Acid)
- Poly. (Succinic Acid)

Weight % Ethanol

Temperature (°F)

Percent by Mass
Relative Proportions

Lactic:Acetic

\[ R^2 = 0.9941 \]

Lactic:Succinic

\[ R^2 = 0.9676 \]
Conclusions

- 120°F is sub-optimal for acid production
- Around 120°F Lactic acid reaches a relative maximum
  - Lactic good, Acetic bad
  - Aim is pH adjustment, not flavor adjustment
What’s the difference?

- Lambic – From the Pajottenland / Senne River Valley Region of Belgium
- American likenesses styled as “American Wild Ale (AWA)” or “American Coolship Ale (ACA)”
- Different Microbial Community
  - Follow same general succession
  - ACA involves a more diverse community of Lactic Acid Bacteria and Minority Yeasts
The Chemistry of Sour Beers

Goals

- Comparative analysis of organic acids using quantitative NMR
  - Manually integrated against a known mass of Maleic Acid
    - Lactic Acid, Acetic Acid, Succinic Acid, Citric Acid, Malic Acid
- Analyze linear and branched dextrin ratios among multiple styles
- Utilize multivariate analysis to discriminate multiple styles of sour beer
Drie Fonteinen Oude Gueuze Straight Run; Assignments from Rodrigues et al. 2010
Freeze dried Run of Drie Fonteinen Oude Gueuze; Assignments from Rodrigues et al. 2010, Rodrigues & Gil 2011, & Nord et al. 2004
## Acid Differences

<table>
<thead>
<tr>
<th>Beer</th>
<th>LA (mg/L)</th>
<th>AA (mg/L)</th>
<th>SA (mg/L)</th>
<th>CA (mg/L)</th>
<th>MA (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>American Geuze-Style</td>
<td>5386.0</td>
<td>1410.0</td>
<td>238.5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>American Framboise-Style</td>
<td>3896.7</td>
<td>2972.1</td>
<td>394.6</td>
<td>3890.7</td>
<td>0</td>
</tr>
<tr>
<td>American Kriek-Style</td>
<td>4682.8</td>
<td>1965.7</td>
<td>423.4</td>
<td>0</td>
<td>3777.8</td>
</tr>
<tr>
<td>Boone Mariage Parfait 2009</td>
<td>4506.5</td>
<td>488.1</td>
<td>217.0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Oude Geuze Vieille</td>
<td>3497.8</td>
<td>454.1</td>
<td>175.2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Geuze Fond Tradition</td>
<td>6807.8</td>
<td>698.6</td>
<td>218.8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Drie Fonteinen A</td>
<td>5137.6</td>
<td>865.9</td>
<td>234.6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Drie Fonteinen B</td>
<td>5389.9</td>
<td>917.7</td>
<td>228.9</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

### American Geuze-Style
- **LA (mg/L):** 5386.0
- **AA (mg/L):** 1410.0
- **SA (mg/L):** 238.5
- **CA (mg/L):** 0
- **MA (mg/L):** 0

### Belgian Geuze
- **LA (mg/L):** 35%
- **AA (mg/L):** 4%
- **SA (mg/L):** 11%
- **CA (mg/L):** 85%

### American Framboise-Style
- **LA (mg/L):** 35%
- **AA (mg/L):** 27%
- **SA (mg/L):** 3%
- **CA (mg/L):** 0

### American Kriek-Style
- **LA (mg/L):** 35%
- **AA (mg/L):** 35%
- **SA (mg/L):** 4%
- **CA (mg/L):** 18%
- **MA (mg/L):** 43%
Dextrin Ratios

Ratio of α(1-4) Maltodextrin to α(1-6) Maltodextrin by Style

- “Degree of Fermentation”
- Dependent on:
  - Strain
  - Style
  - Ingredients
Multivariate Analysis – Principal Component Analysis

- Segregate based largely on sugar detail
- Independent of ingredients
Conclusions

- ACAs & Belgian Lambics have differing acid profiles
- Ratio of linear and branched maltodextrins can be used to differentiate styles
- Multivariate analysis can differentiate between ACAs, Belgian Lambics and 6 other styles
References