When Beer Goes Sour: An NMR Investigation

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http://www.1zoom.net/Food/wallpaper/316156/z2586.3/

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[2]
    - Modern Lambic – Brasserie Cantillon recipe – 65% Barley Malt + 35% Raw Wheat[2]
- 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.newbelgium.com/Community/Blog/12-03-23/Who-wants-more-sour-beer.aspx

http://www.belgianbeermagazine.com/oud-beersel-brewery/

Who goes in to a sour beer?

- Dozens of organisms[2,3]
  - Bacteria
    - *Enterobacteriaceae*
      - *Citrobacter* spp., *Enterobacter* spp., *Klebsiella* spp., *Hafnia* spp.
    - *Lactobacillaceae*
      - *Pediococcus* spp., *Lactobacillus* spp.,
    - *Acetobacter* spp.
    - *Klebsiella* spp.
  - Yeasts
    - *Kloeckera apiculata*
    - *Saccharomyces* spp.
    - *Brettanomyces* spp.
    - *Pichia* spp.
    - *Candida* spp.
    - *Hansenula* spp.
    - *Cryptococcus* spp.

Why is Sour Beer Sour?

- Straight Lambic, Flanders Ales, Gueuze, Berliner Weisse
  - Lactic, Acetic, Succinic Acid[1]
    - 85% - 10% - 5%
- Fruit Lambics[1]
  - 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

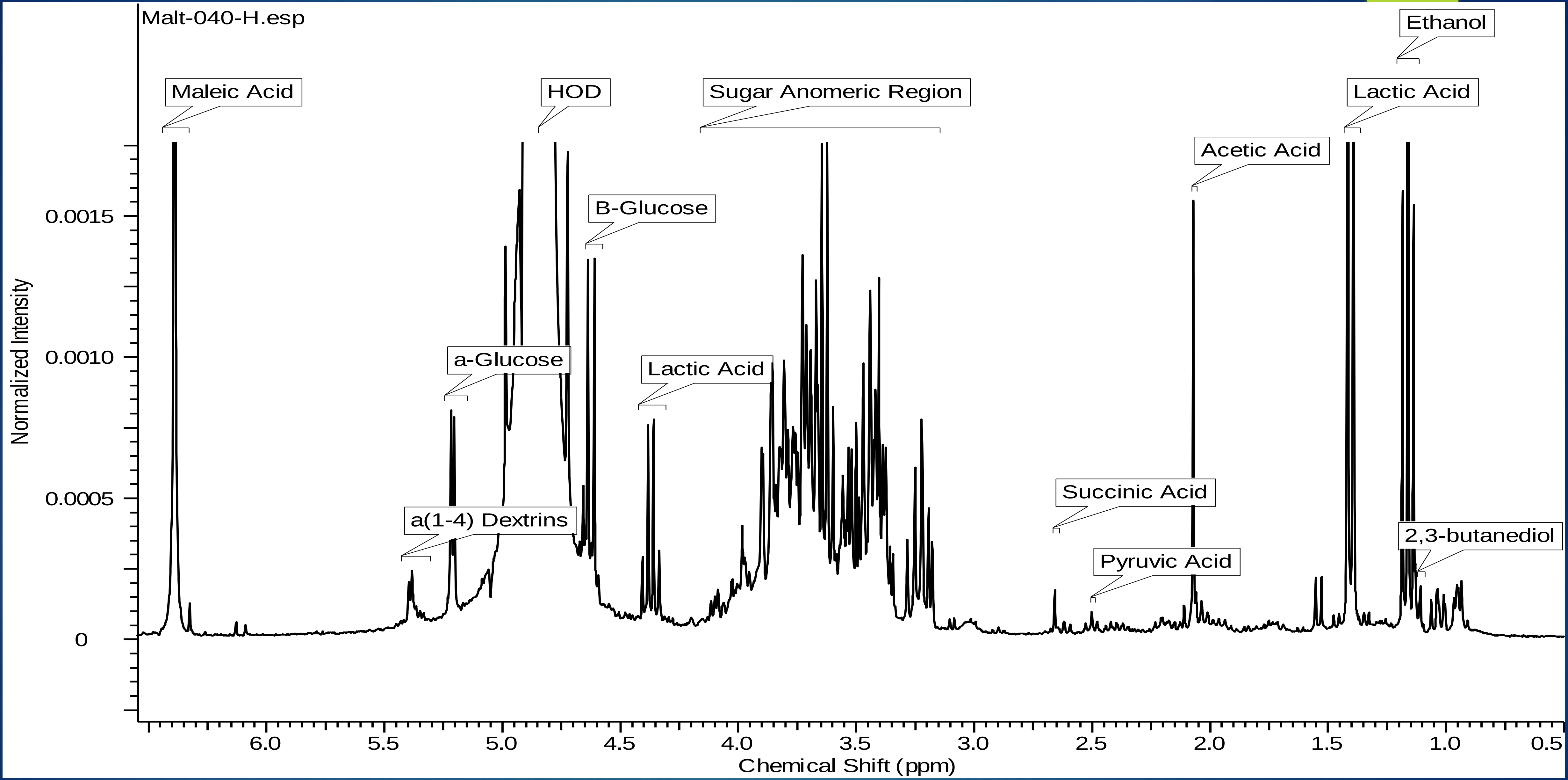
Berliner Weisse & Biological Acidification

- Reinheitsgebot of 1516
  - Beer can contain only malt, hops & water
    - Unmalted wheat and yeast added in the Provisional Law of 1996[4]
  - Artificial alteration of pH is illegal[5]
    - Development of Biological Acidification/Sour Mashing
      - Utilization of native microbes for pH adjustment[6]

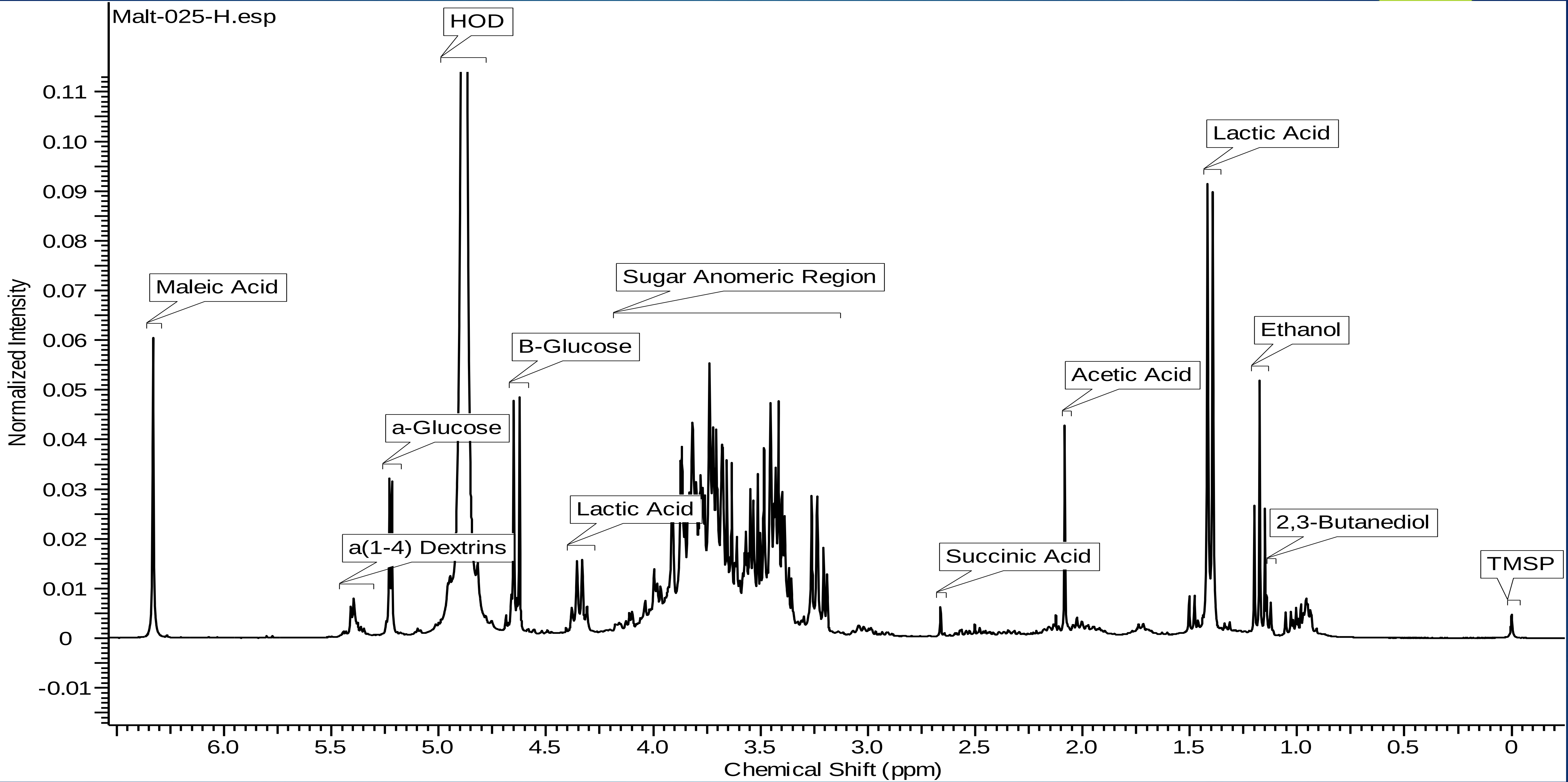
Perform Starch Conversion → Cool to ~120°F → Innoculate → Hold Temperature (120°F)

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
      - γ-Amino Butyric Acid (contaminant)

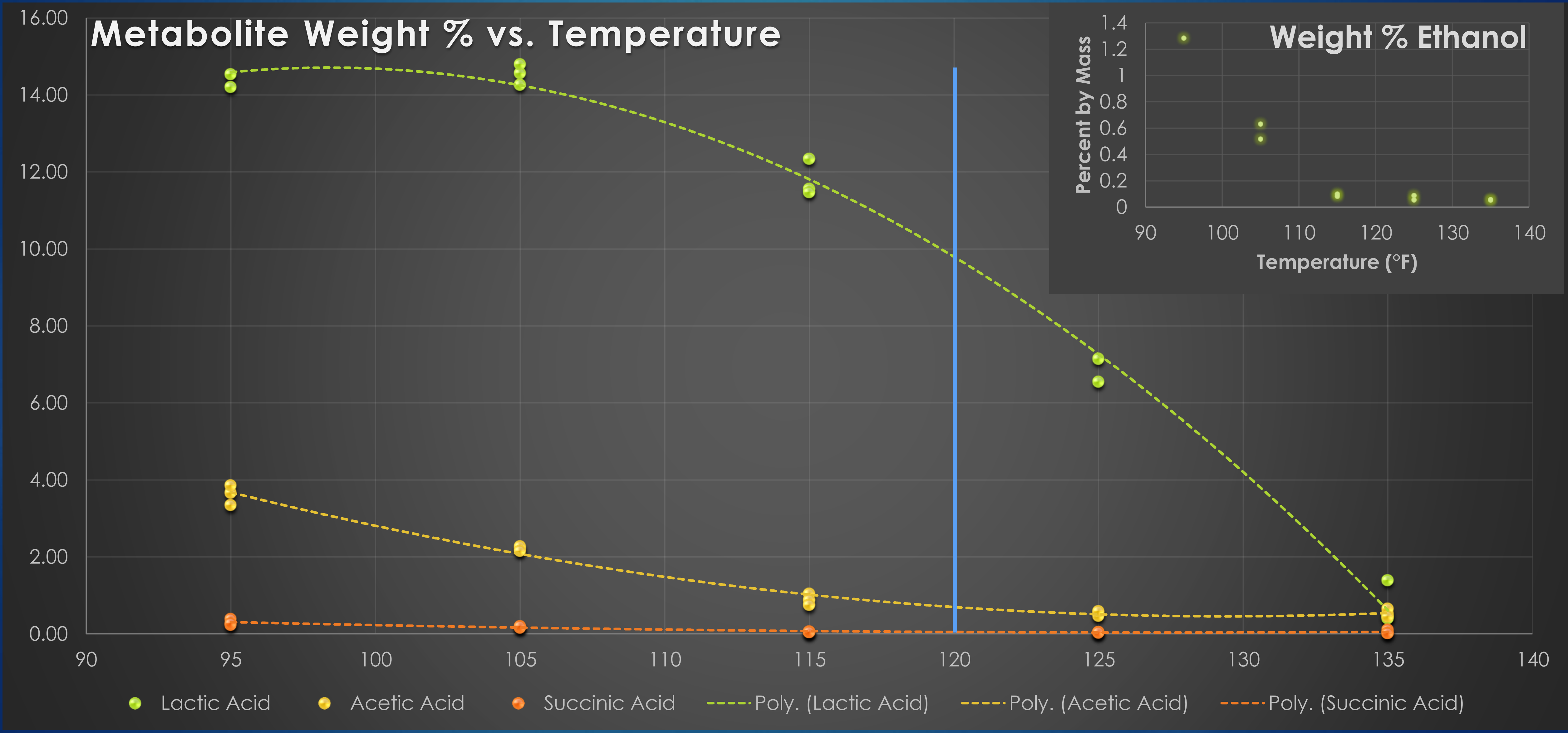


- Straight Run of 95°F Sour mash; Assignments from Rodrigues et al. 2010

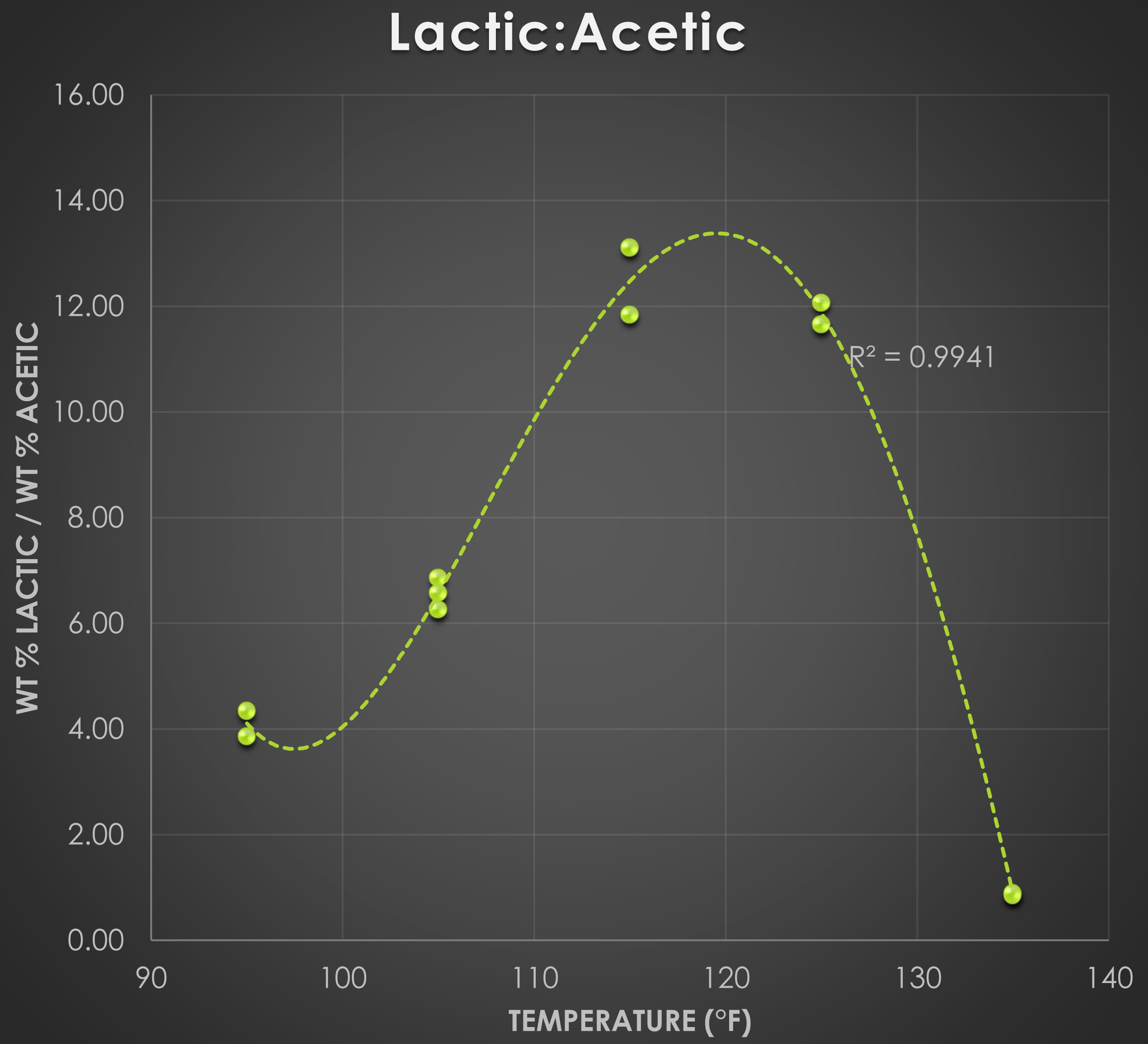


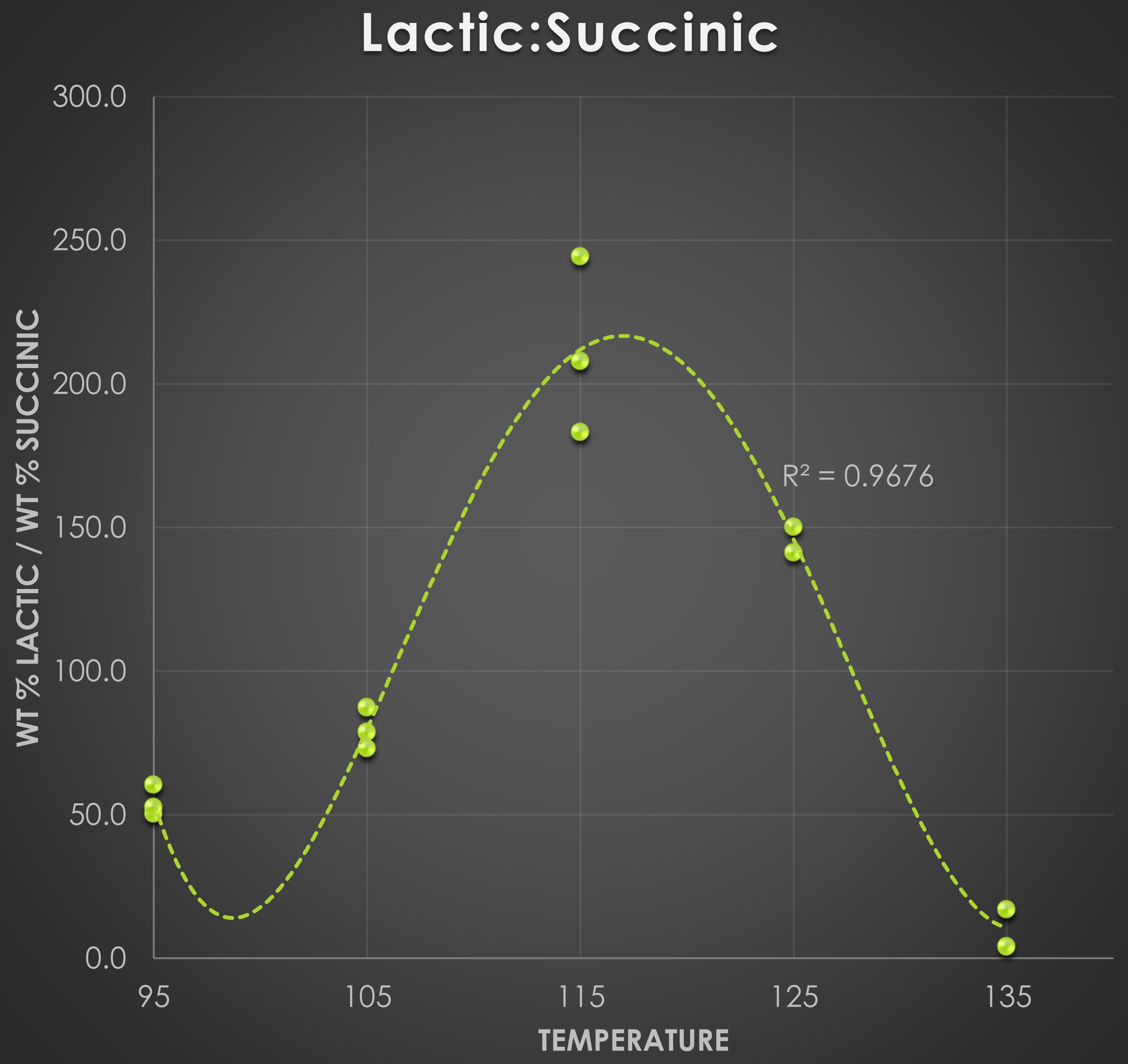
- Freeze Dried Run of 95°F sour Mash; Assignments from Rodrigues et al. 2010

Absolute Metabolite Proportions



Relative Proportions



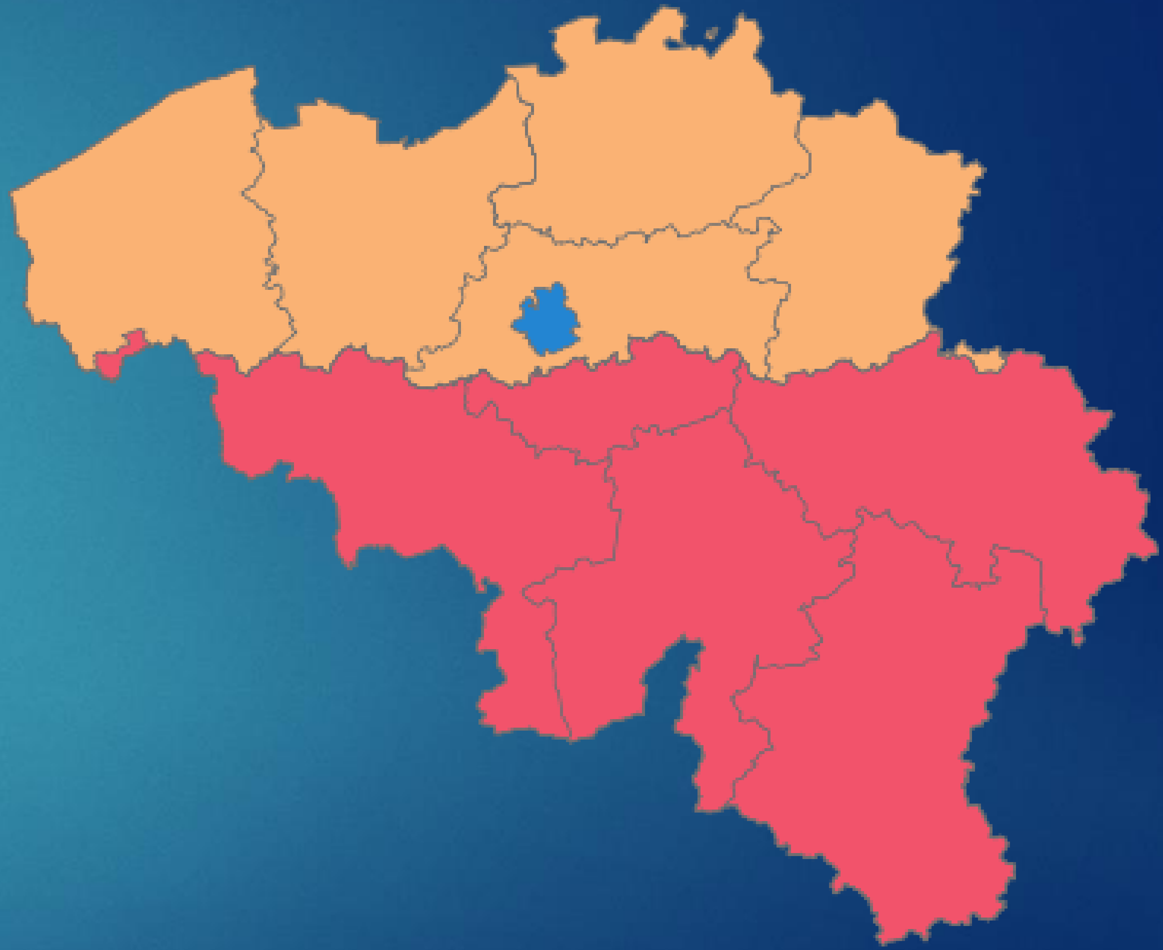


Conclusions

- 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

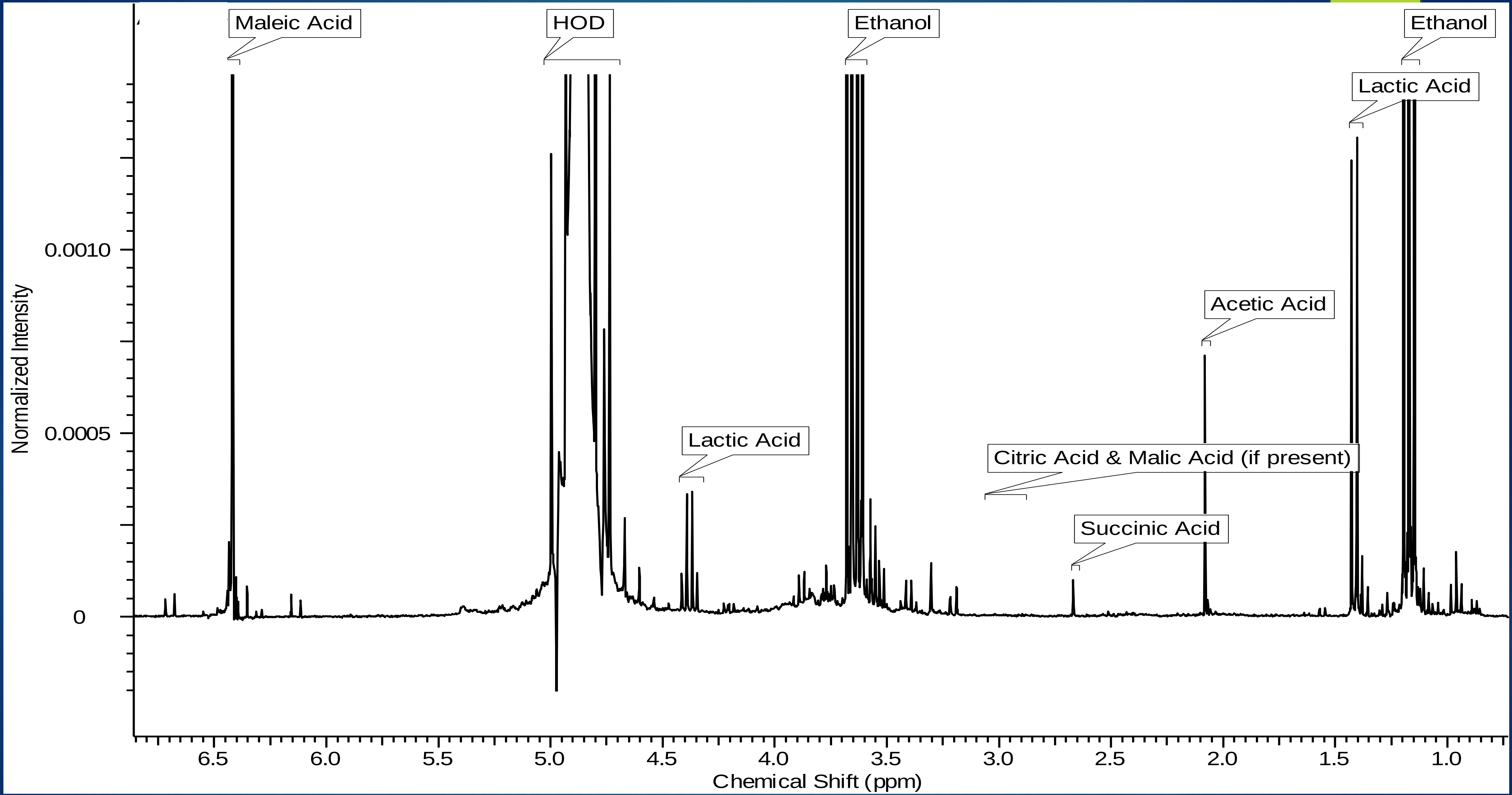
Lambics of Belgium & Lambic-Styles of the USA

- 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[3]

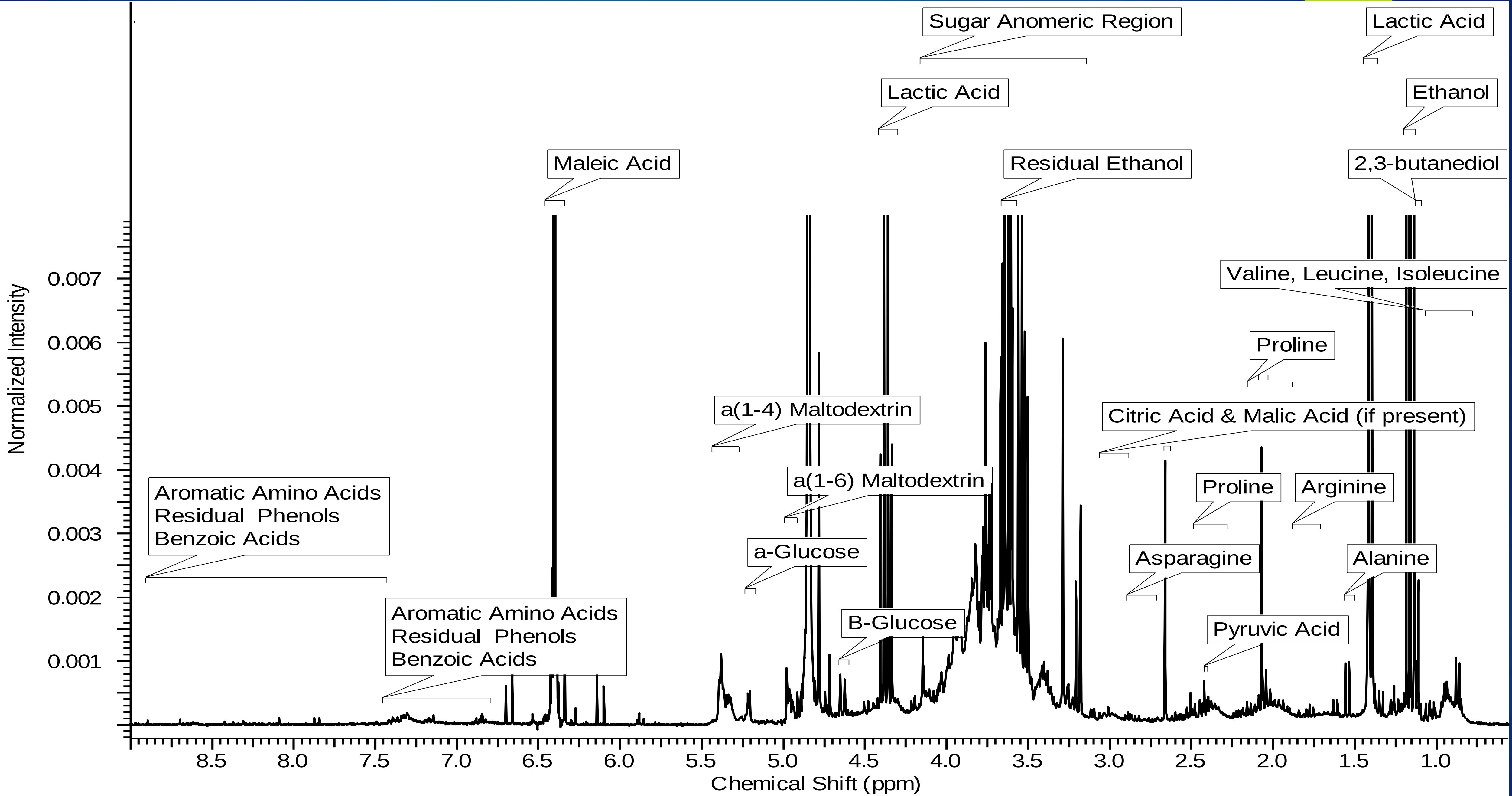


The Chemistry of Sour Beers

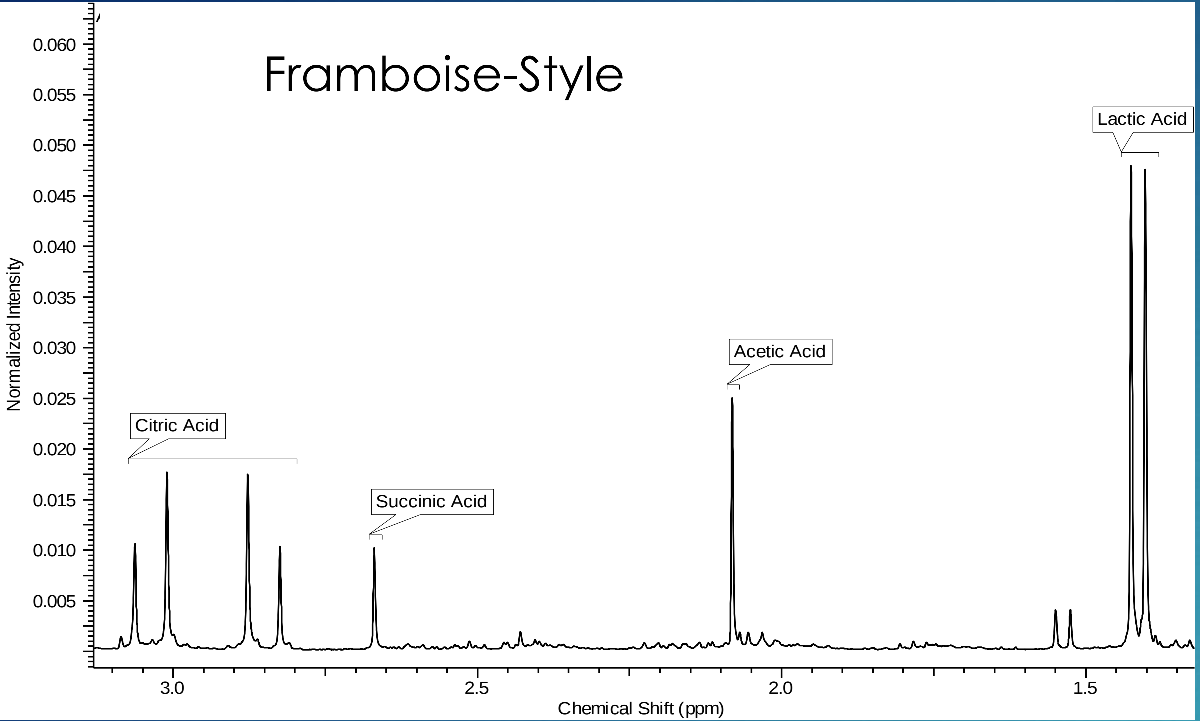
- Goals
  - Comparative analysis of organic acids using quantitative NMR
    - Manually integrated against a known mass of Maleic Acid
      - Lactic Acid, Acetic Acid, Succinic Acid, Citric Acid, Malic Acid
  - Analyze linear and branched dextrin ratios among multiple styles
  - Utilize multivariate analysis to discriminate multiple styles of sour beer

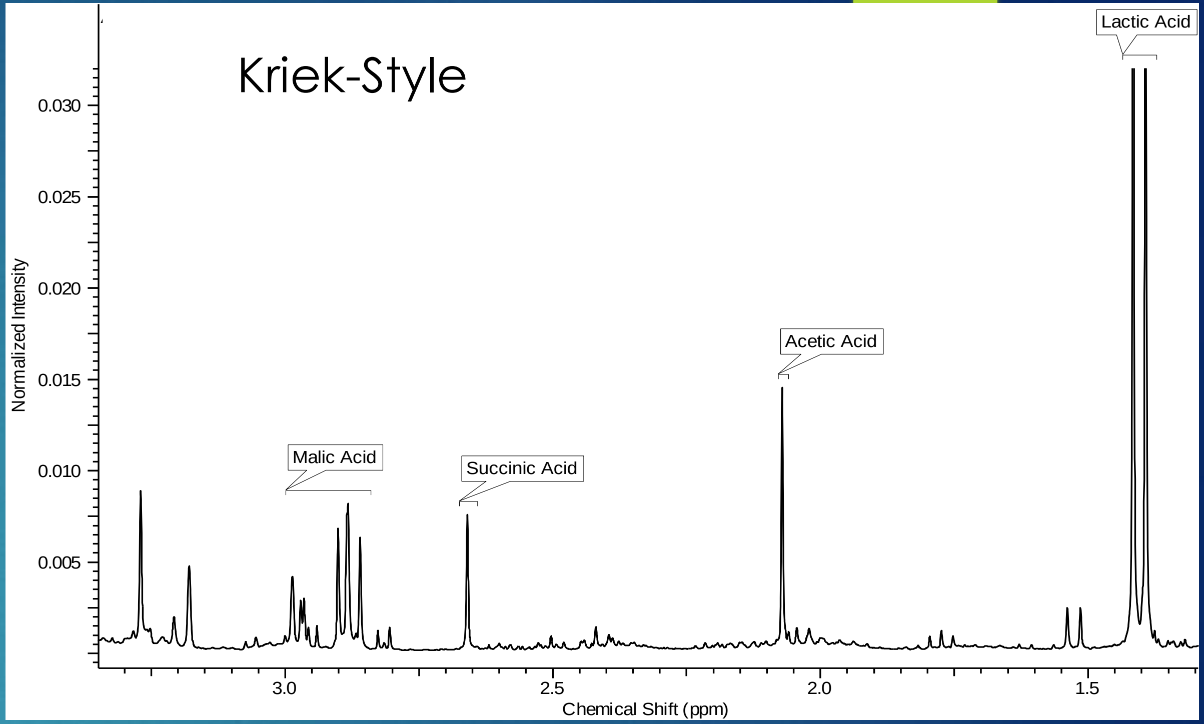


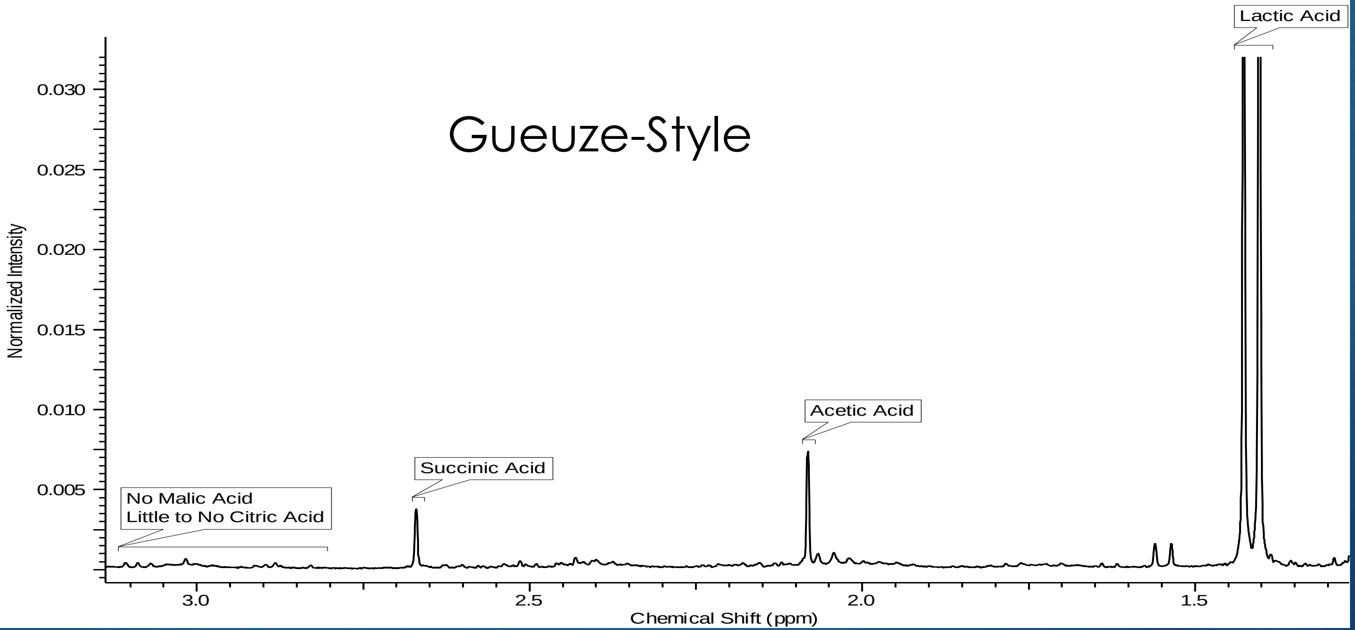
- Drie Fonteinen Oude Gueuze Straight Run; Assignments from Rodrigues et al. 2010



- Freeze dried Run of Drie Fonteinen Oude Gueuze; Assignments from Rodrigues et al. 2010, Rodrigues & Gil 2011, & Nord et al. 2004

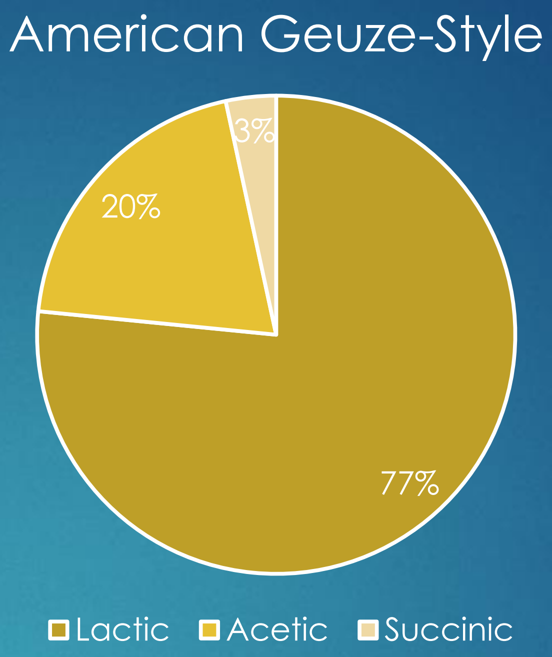

Framboise-Style
Citric Acid
Succinic Acid
Acetic Acid
Lactic Acid
Normalized Intensity
Chemical Shift (ppm)
0.060
0.055
0.050
0.045
0.040
0.035
0.030
0.025
0.020
0.015
0.010
0.005
3.0
2.5
2.0
1.5

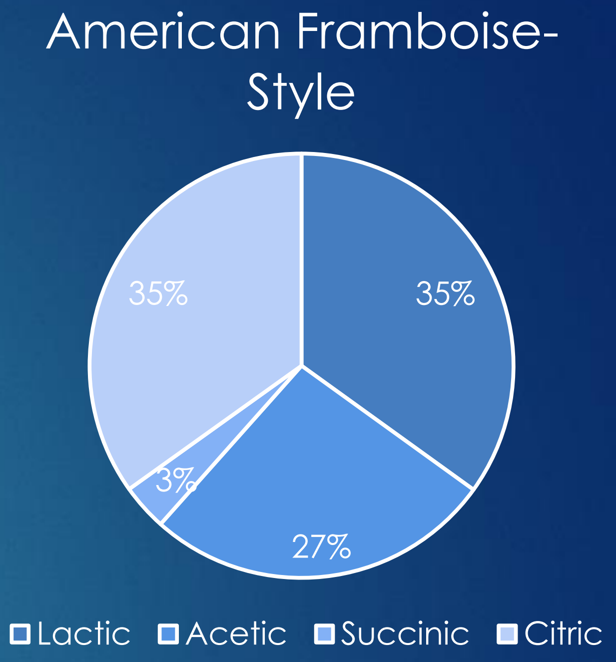

Kriek-Style
Lactic Acid
Acetic Acid
Malic Acid
Succinic Acid
Normalized Intensity
Chemical Shift (ppm)
0.030
0.025
0.020
0.015
0.010
0.005
3.0
2.5
2.0
1.5

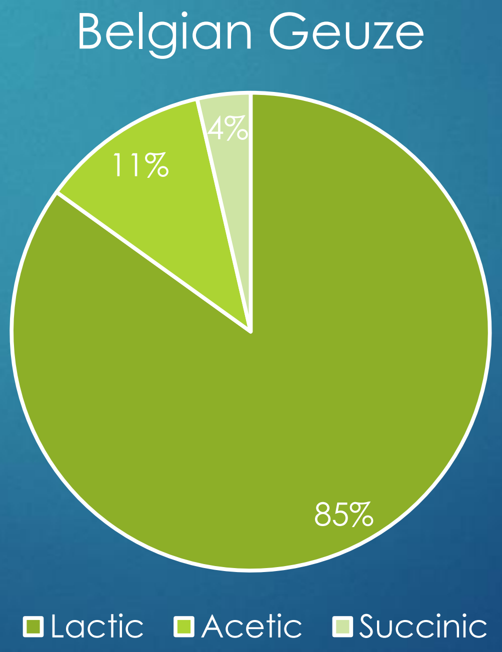

Gueuze-Style
Lactic Acid
Acetic Acid
Succinic Acid
No Malic Acid
Little to No Citric Acid
Normalized Intensity
Chemical Shift (ppm)
0.030
0.025
0.020
0.015
0.010
0.005
3.0
2.5
2.0
1.5

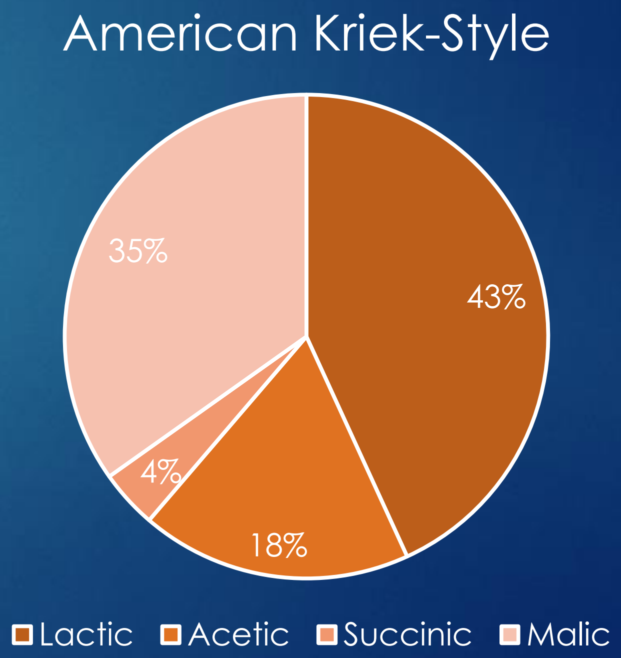
Acid Differences

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

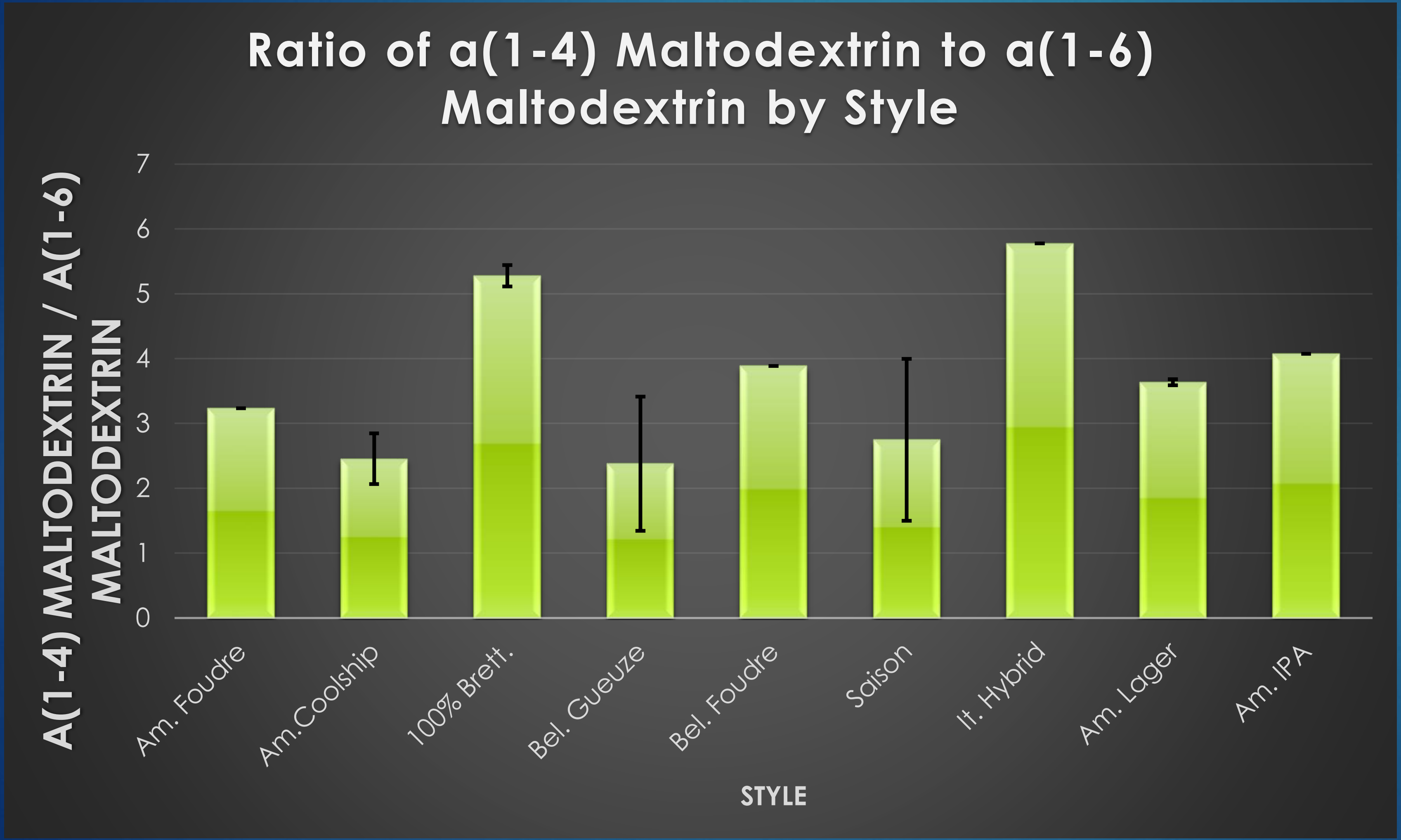






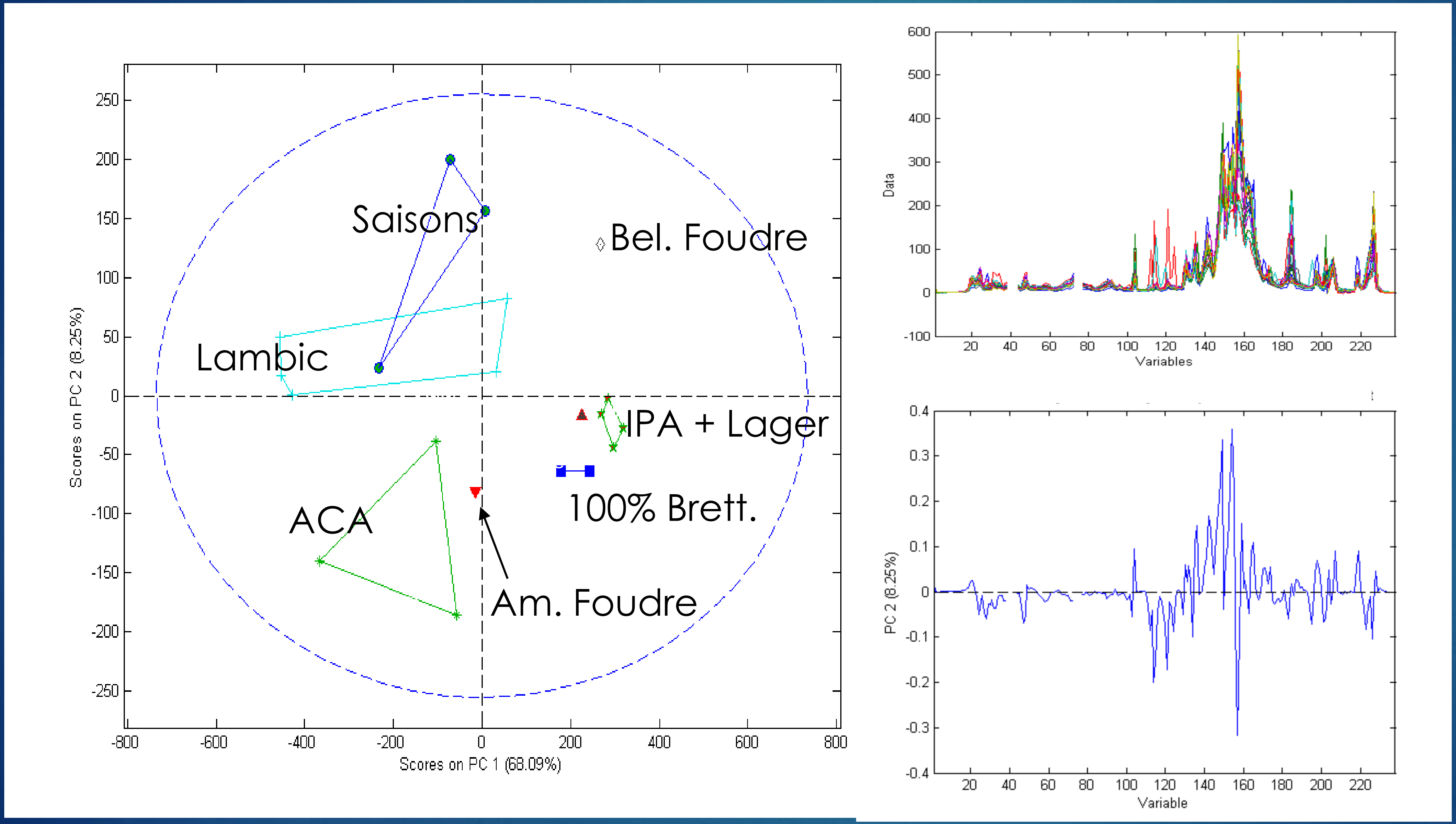


Dextrin Ratios



- "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

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9. Nord, L. I., Vaag, P., Duus, J. O. (2004). Quantification of Organic and Amino Acids in Beer by 1H NMR Spectroscopy, *Anal. Chem.* 76(16):4790-4798.