When Beer Goes Sour: An NMR Investigation

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MESTRELAB MNOVA USERS MEETING

SMASH – ATLANTA, GA, SEPTEMBER 7, 2014

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
  - 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.*

Typical Coolship design.

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

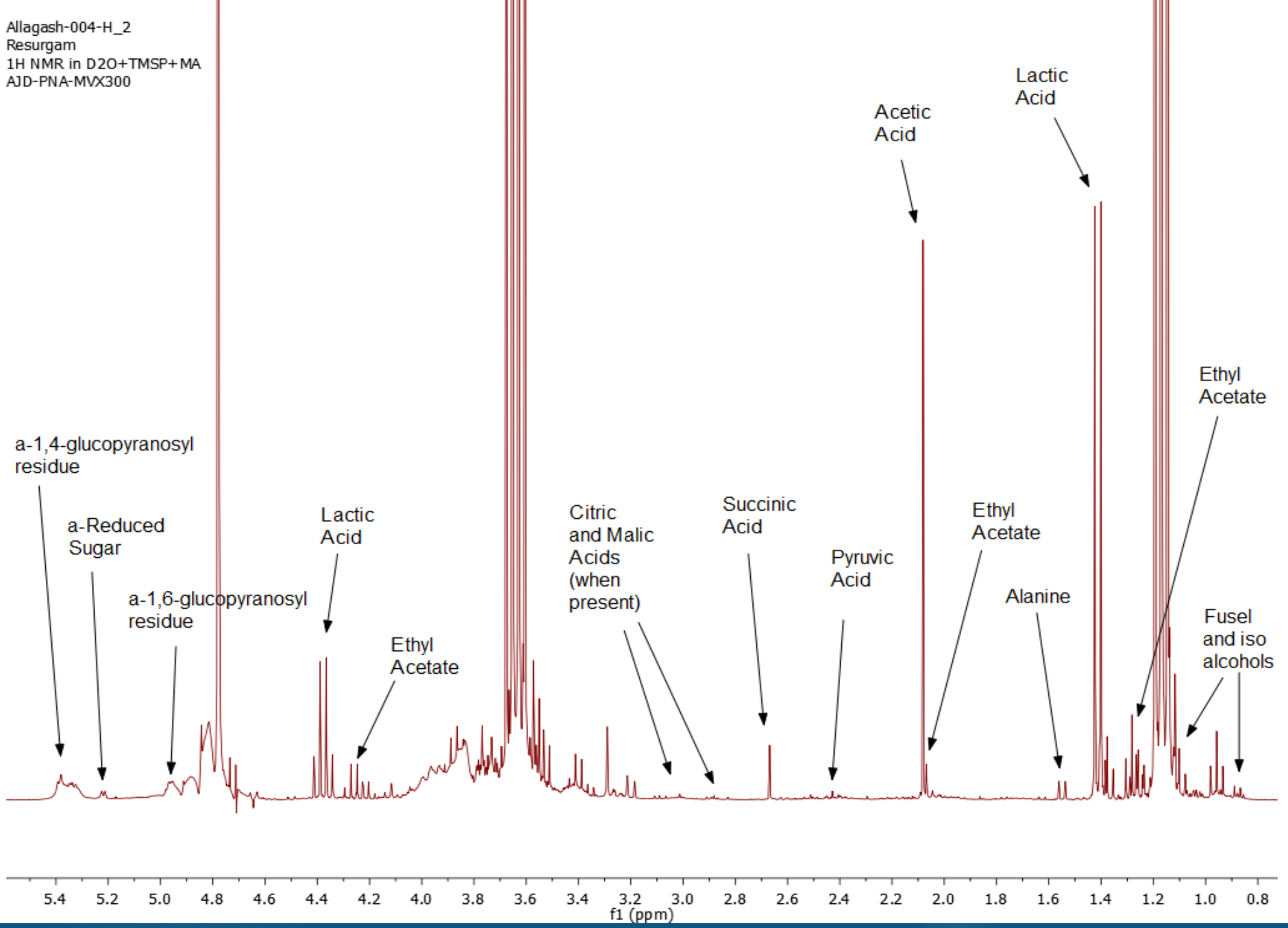
Berliner Weisse & Biological Acidification

- 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]

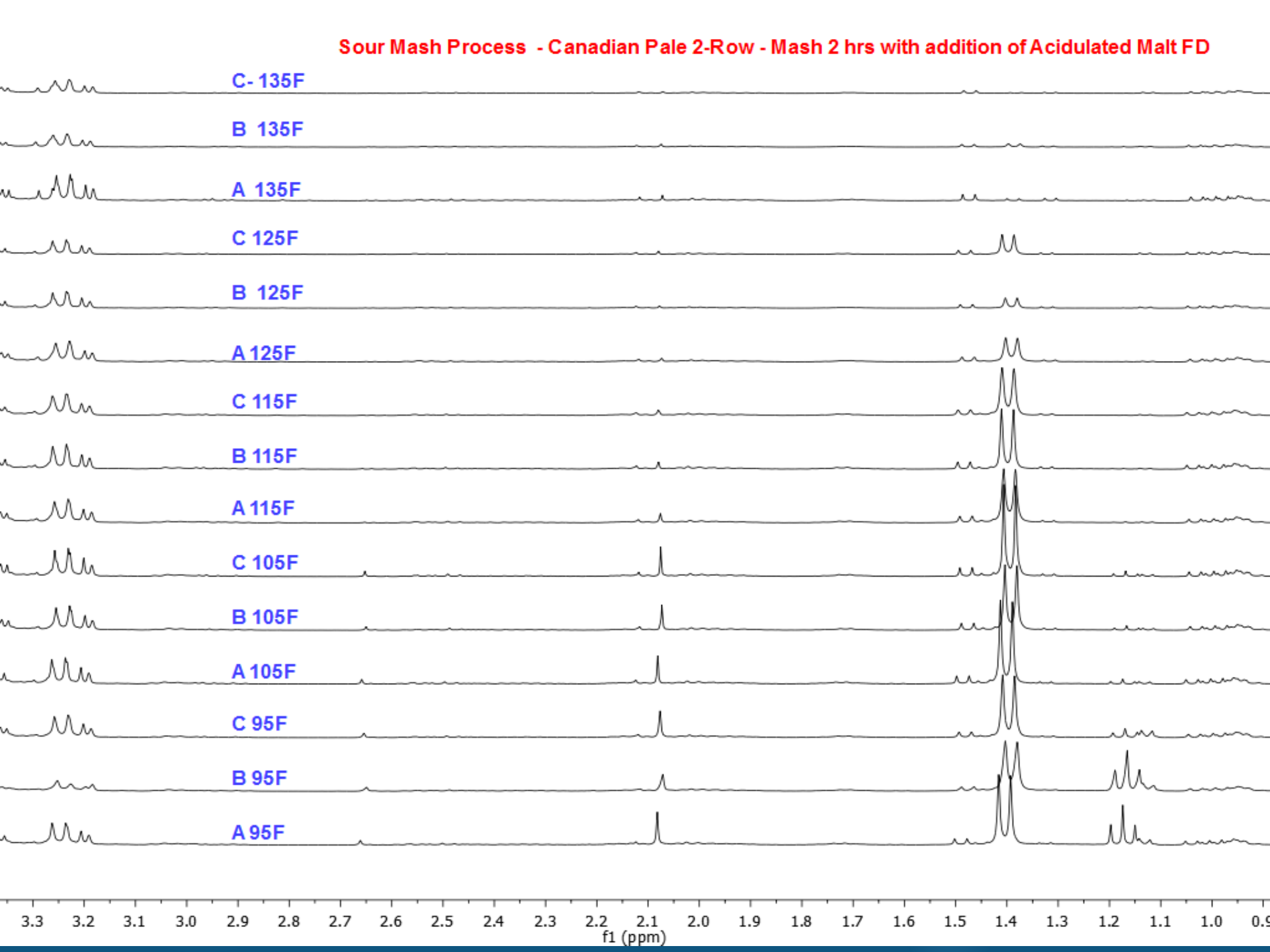
Temperature Dependence of the Sour Mash Technique

- 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

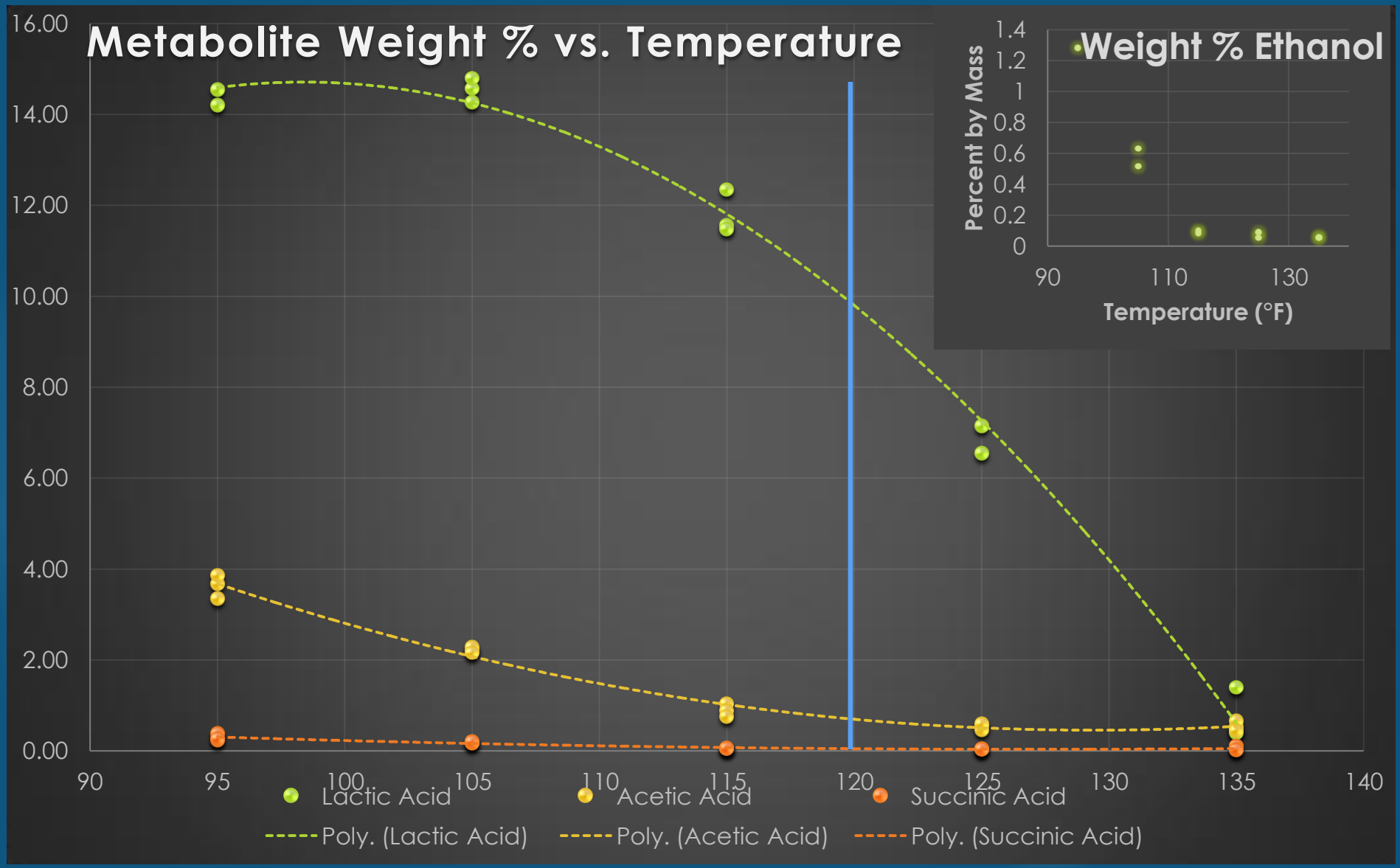
      manual integration, and Mestrelab GSD SMA plug-in
      - Lactic Acid
      - Acetic Acid (contaminant)
      - Succinic Acid
      - Ethanol
      - γ-Amino Butyric Acid (contaminant)



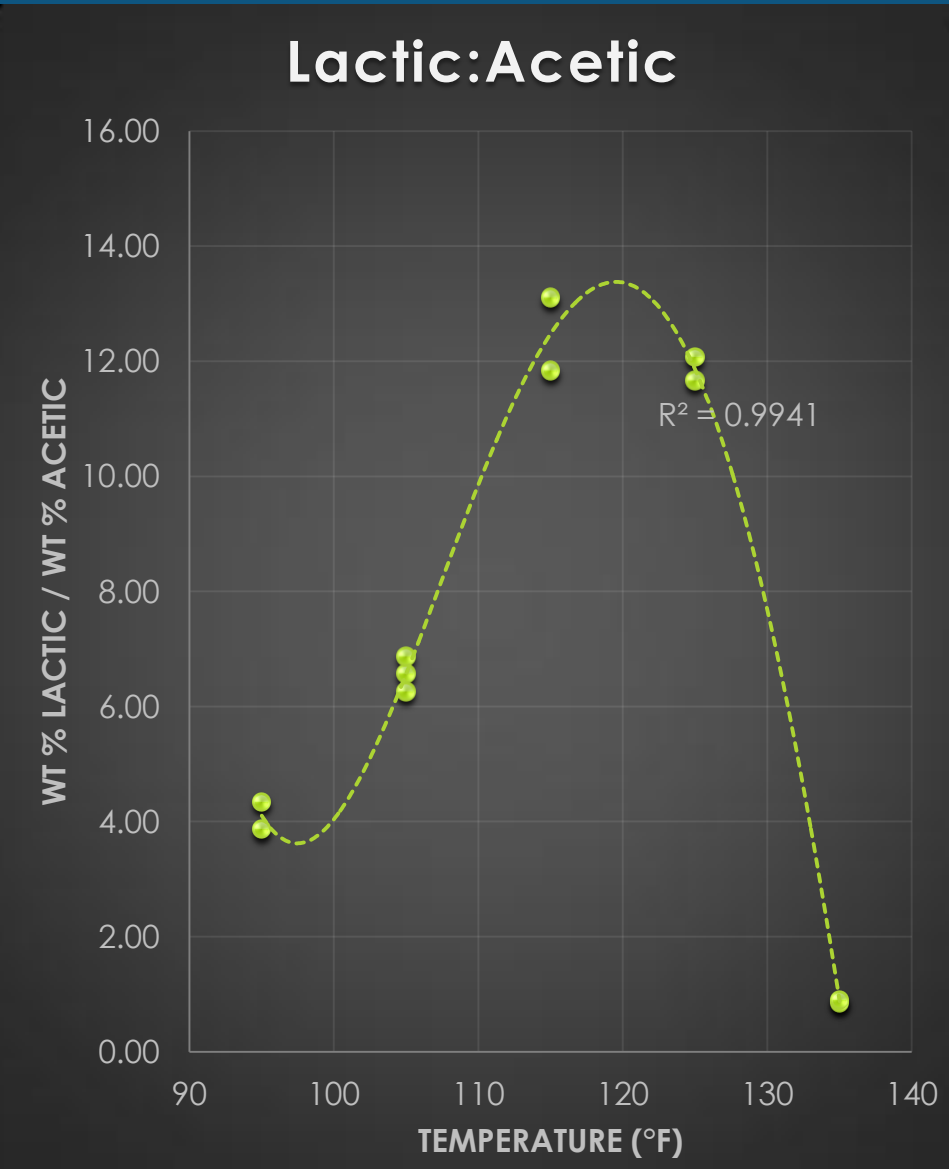
Typical beer chemistry observed by ^{1}H NMR

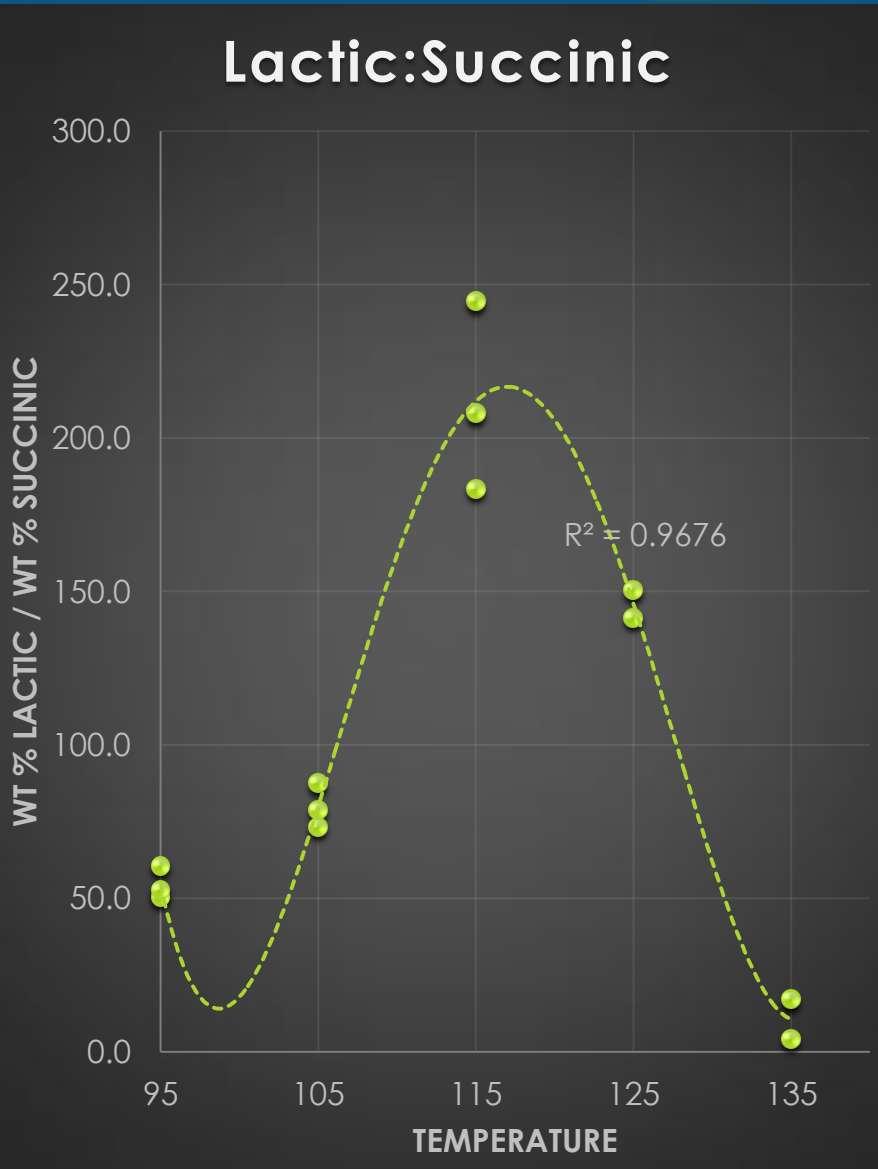

Sour Mash Process - Canadian Pale 2-Row - Mash 2 hrs with addition of Acidulated Malt FD
C- 135F
B 135F
A 135F
C 125F
B 125F
A 125F
C 115F
B 115F
A 115F
C 105F
B 105F
A 105F
C 95F
B 95F
A 95F
3.3 3.2 3.1 3.0 2.9 2.8 2.7 2.6 2.5 2.4 2.3 2.2 2.1 2.0 1.9 1.8 1.7 1.6 1.5 1.4 1.3 1.2 1.1 1.0 0.9
f1 (ppm)

Absolute Metabolite Proportions



Relative Proportions



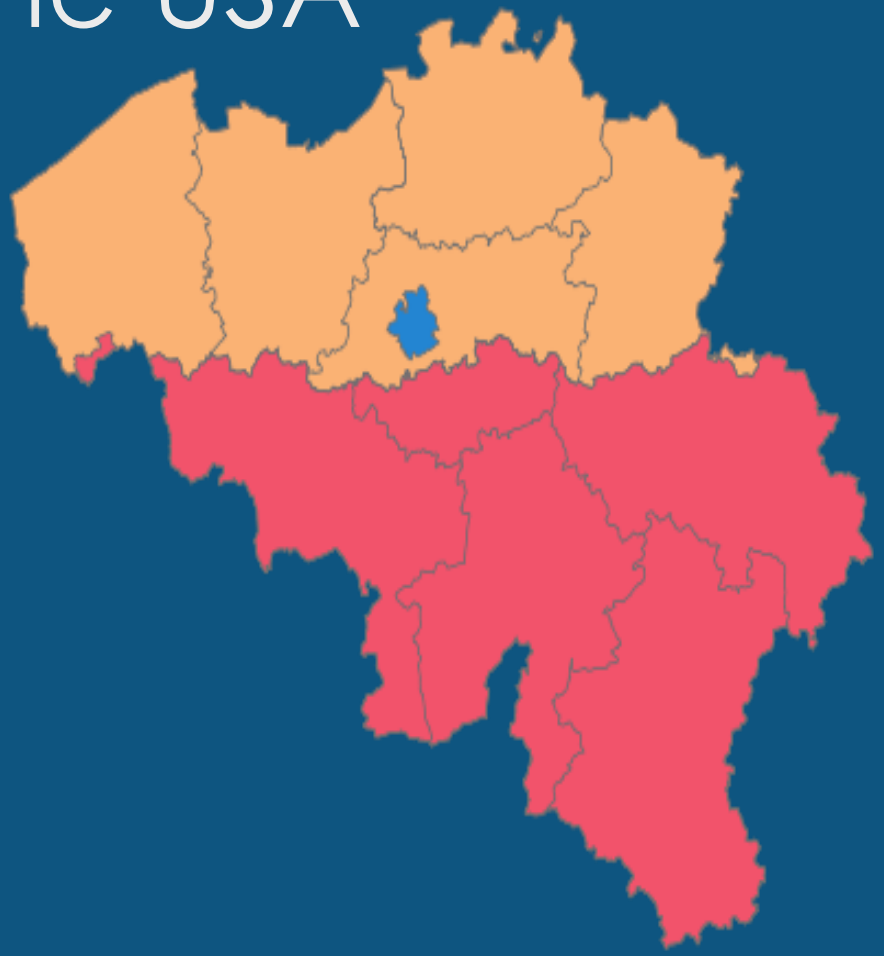


Conclusions

- 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

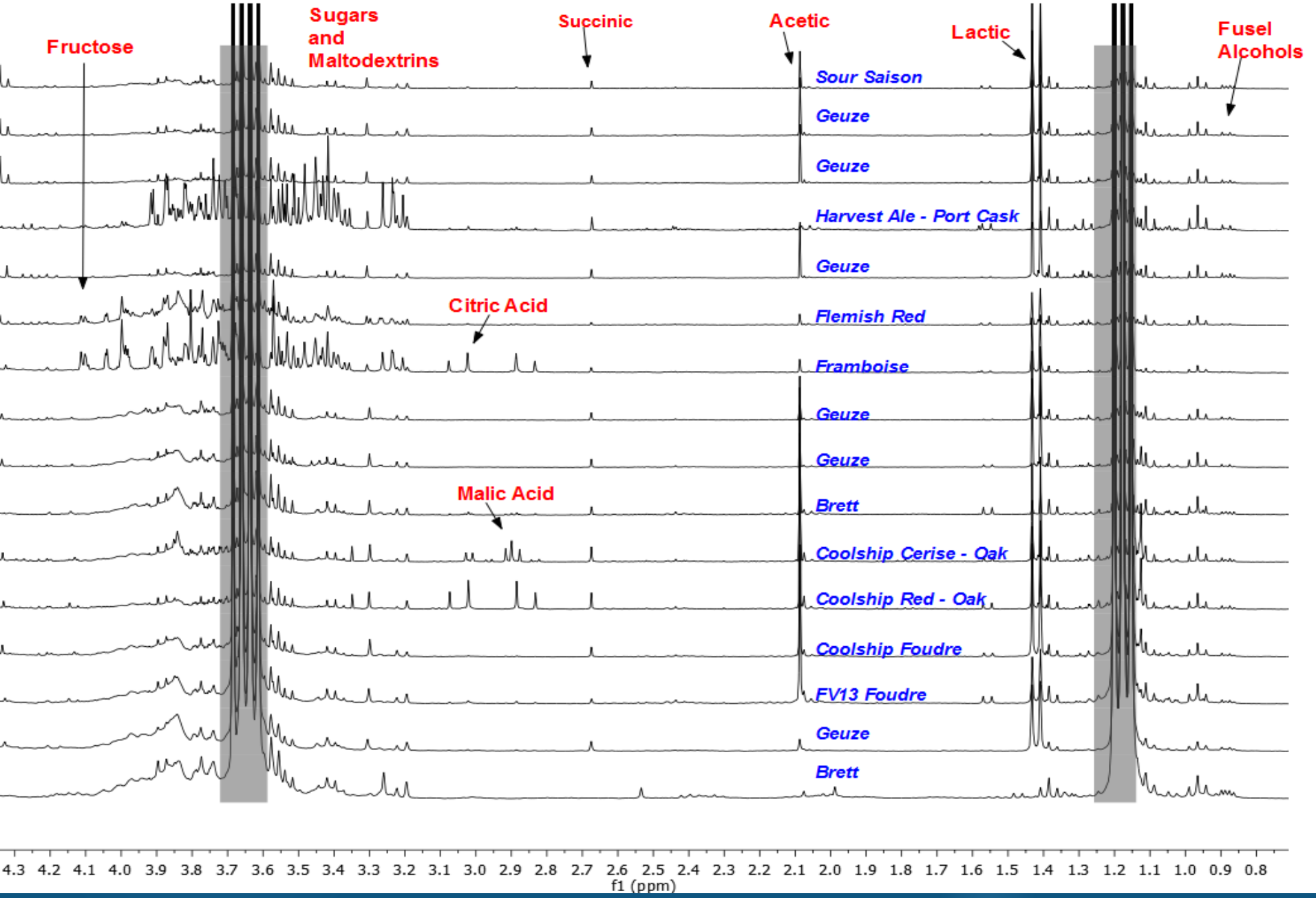
Lambics of Belgium & Lambic-Styles of the USA

- 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



The Chemistry of Sour Beers

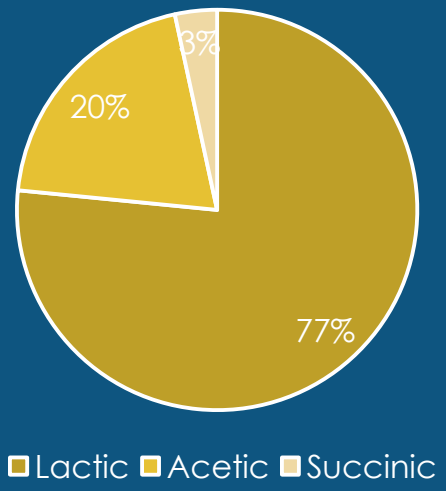
- Goals
  - Comparative analysis of organic acids using quantitative NMR
    - Manually integrated and SMA analysis against a known mass of Maleic Acid
      - Lactic Acid, Acetic Acid, Succinic Acid, Citric Acid, Malic Acid
  - Analyze linear and branched dextrin ratios among multiple styles
  - Utilize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


Fructose
Sugars and Maltodextrins
Succinic
Acetic
Lactic
Fusel Alcohols
Sour Saison
Geuze
Geuze
Harvest Ale - Port Cask
Geuze
Citric Acid
Flemish Red
Framboise
Geuze
Geuze
Malic Acid
Brett
Coolship Cerise - Oak
Coolship Red - Oak
Coolship Foudre
FV13 Foudre
Geuze
Brett
4.3 4.2 4.1 4.0 3.9 3.8 3.7 3.6 3.5 3.4 3.3 3.2 3.1 3.0 2.9 2.8 2.7 2.6 2.5 2.4 2.3 2.2 2.1 2.0 1.9 1.8 1.7 1.6 1.5 1.4 1.3 1.2 1.1 1.0 0.9 0.8
f1 (ppm)

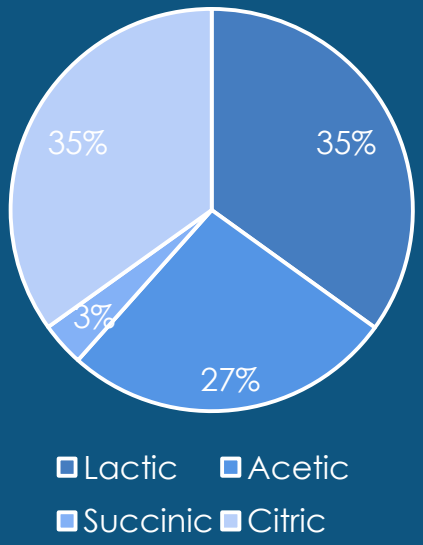
Acid Differences

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

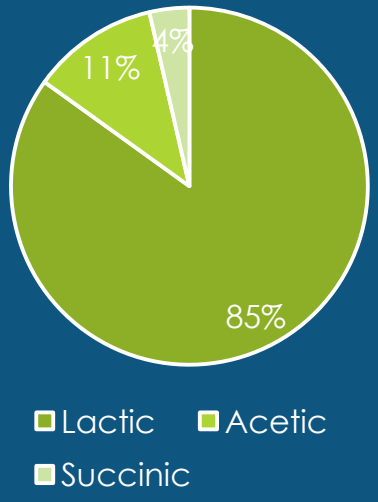
American Geuze-Style



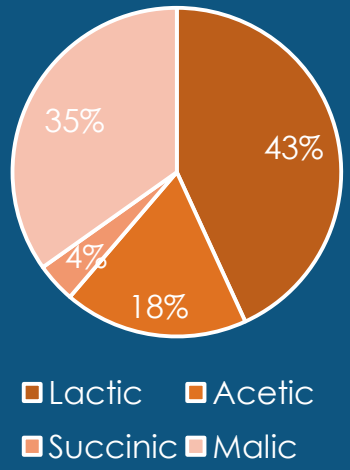
American Framboise-Style



Belgian Geuze

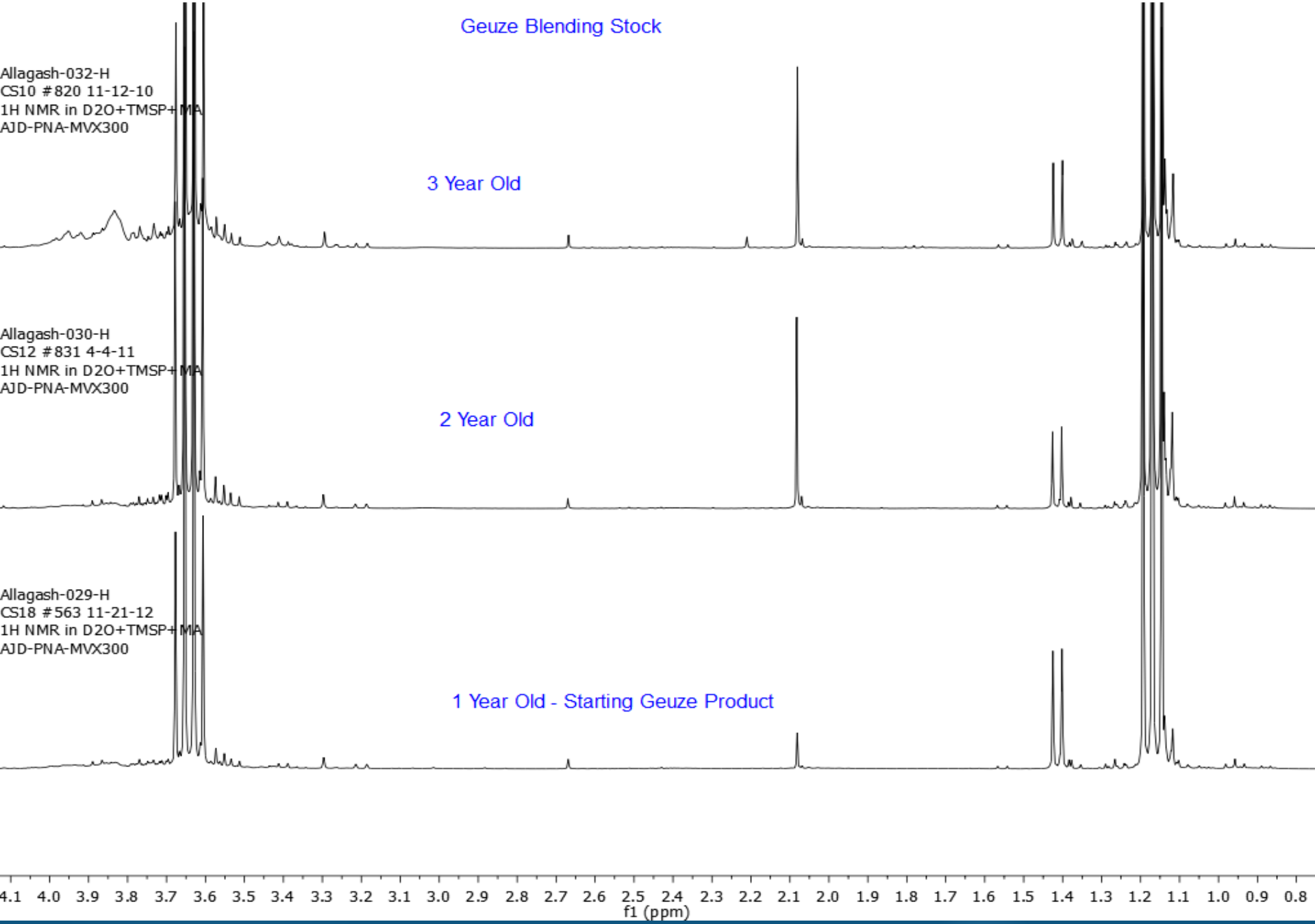


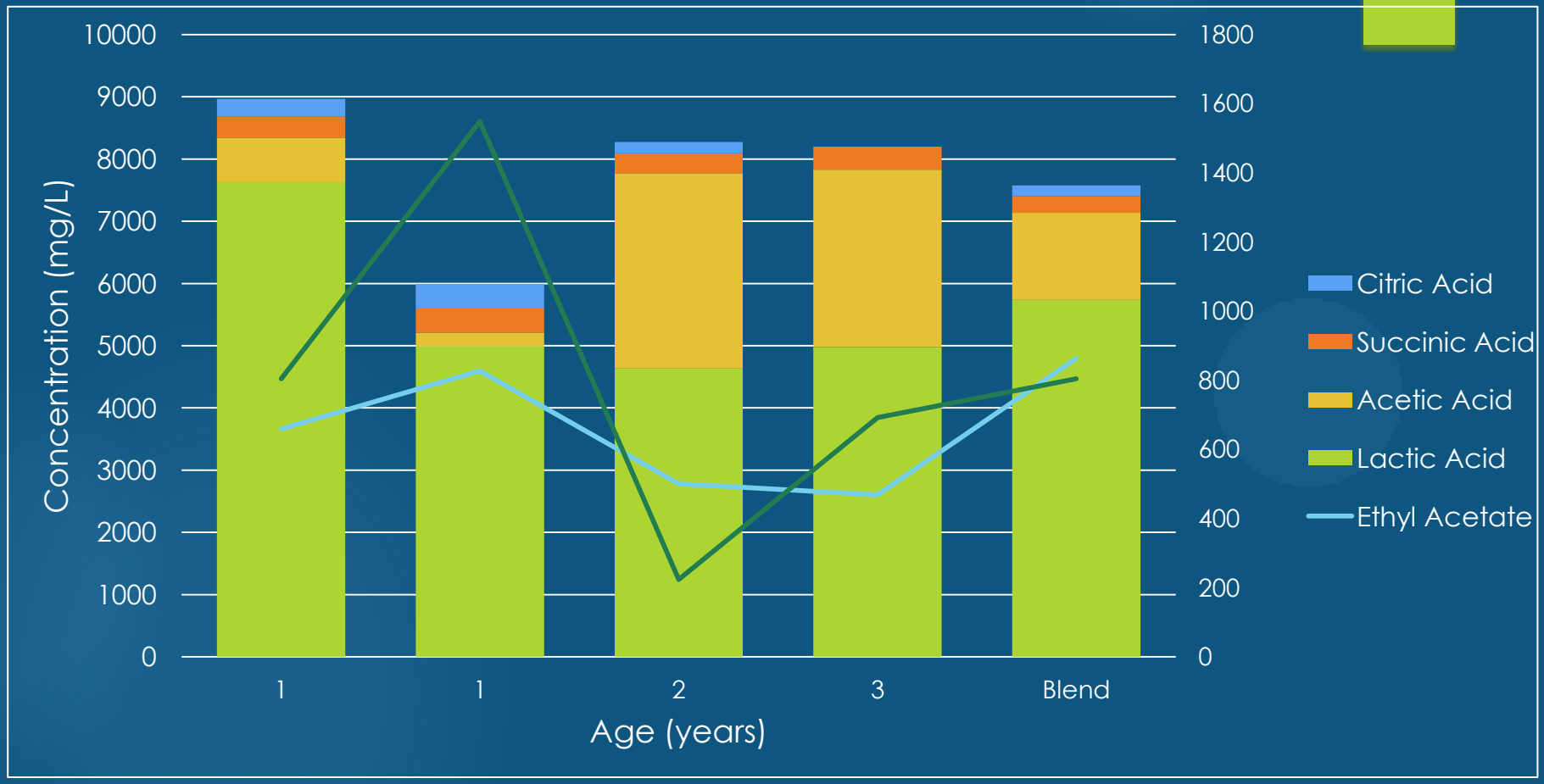
American Kriek-Style



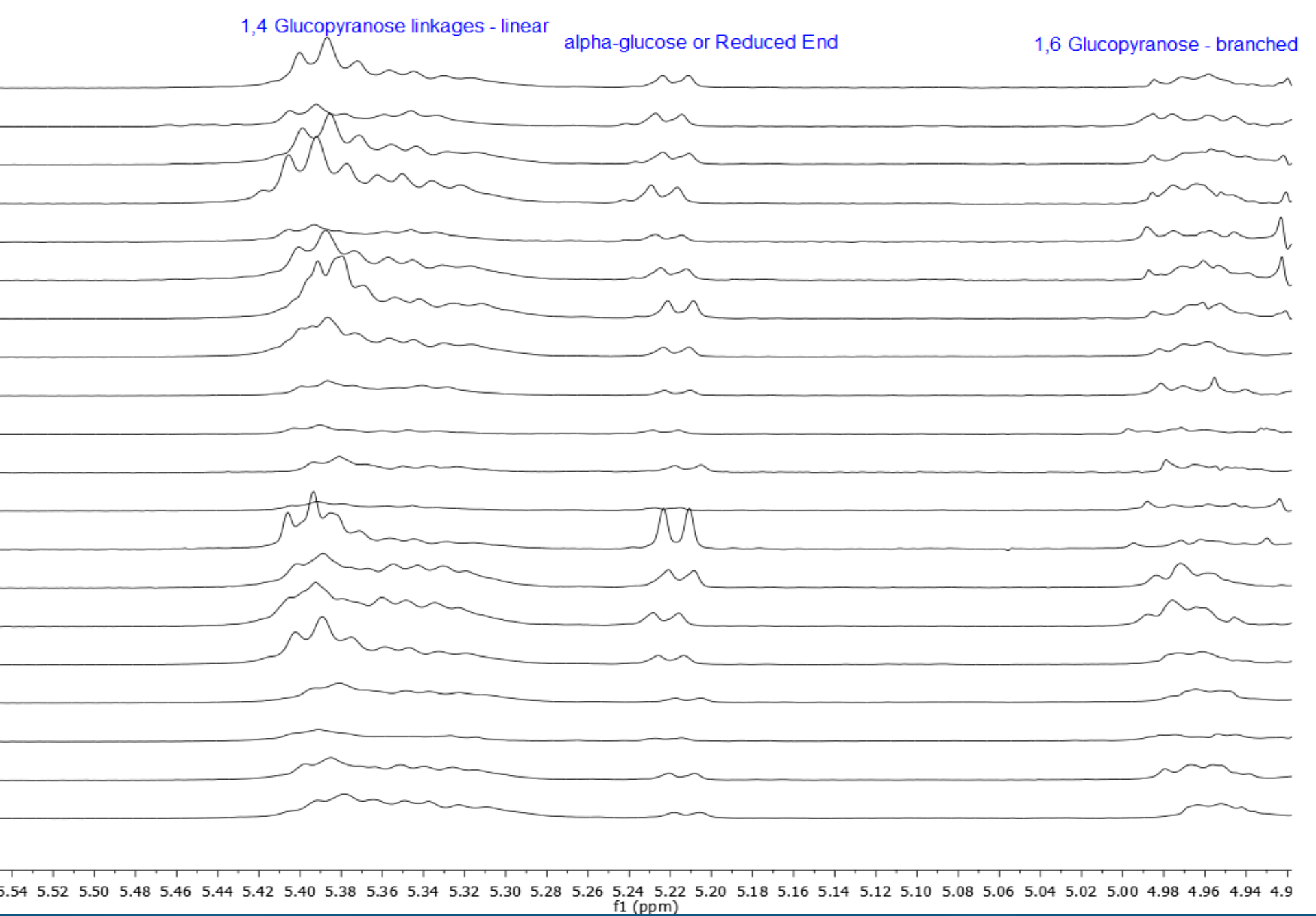
Comparison of results obtained by manual integration and by automatic Mnova SMA. Units=mg/L

		Manual Integration				Mnova SMA			
Brewery	Beer	Lactic Acid	Acetic Acid	Succinic Acid	Citric Acid	Lactic Acid	Acetic Acid	Succinic Acid	Citric Acid
Allagash	Confluence	850.5	402.4	167.4	248.2	901.3	437.6	184.3	353.2
Budweiser	Bud Light	93.9	38.1	35.8	82.7	117.1	17.7	34.2	62.6
Crooked Stave	Surette	4699.2	564.9	195.7	265.5	4247.9	549.2	185.6	262.4
Drie Fonteinen	Oude Gueuze	5137.6	865.9	234.6	0.0	4935.2	787.1	204.6	0.0
Drie Fonteinen	Oude Gueuze	5389.9	917.7	228.9	0.0	5180.0	775.2	194.4	0.0
LoverBeer	D'Uva Beer	3071.4	781.5	876.0	358.0	3302.8	759.9	842.4	377.3
Boone	Mariage Parfait 2009	4506.5	488.1	216.9	0.0	4591.8	403.2	193.1	0.0


Geuze Blending Stock
Allagash-032-H
CS10 #820 11-12-10
1H NMR in D2O+TMSP+MA
AJD-PNA-MVX300
3 Year Old
Allagash-030-H
CS12 #831 4-4-11
1H NMR in D2O+TMSP+MA
AJD-PNA-MVX300
2 Year Old
Allagash-029-H
CS18 #563 11-21-12
1H NMR in D2O+TMSP+MA
AJD-PNA-MVX300
1 Year Old - Starting Geuze Product
4.1 4.0 3.9 3.8 3.7 3.6 3.5 3.4 3.3 3.2 3.1 3.0 2.9 2.8 2.7 2.6 2.5 2.4 2.3 2.2 2.1 2.0 1.9 1.8 1.7 1.6 1.5 1.4 1.3 1.2 1.1 1.0 0.9 0.8
f1 (ppm)

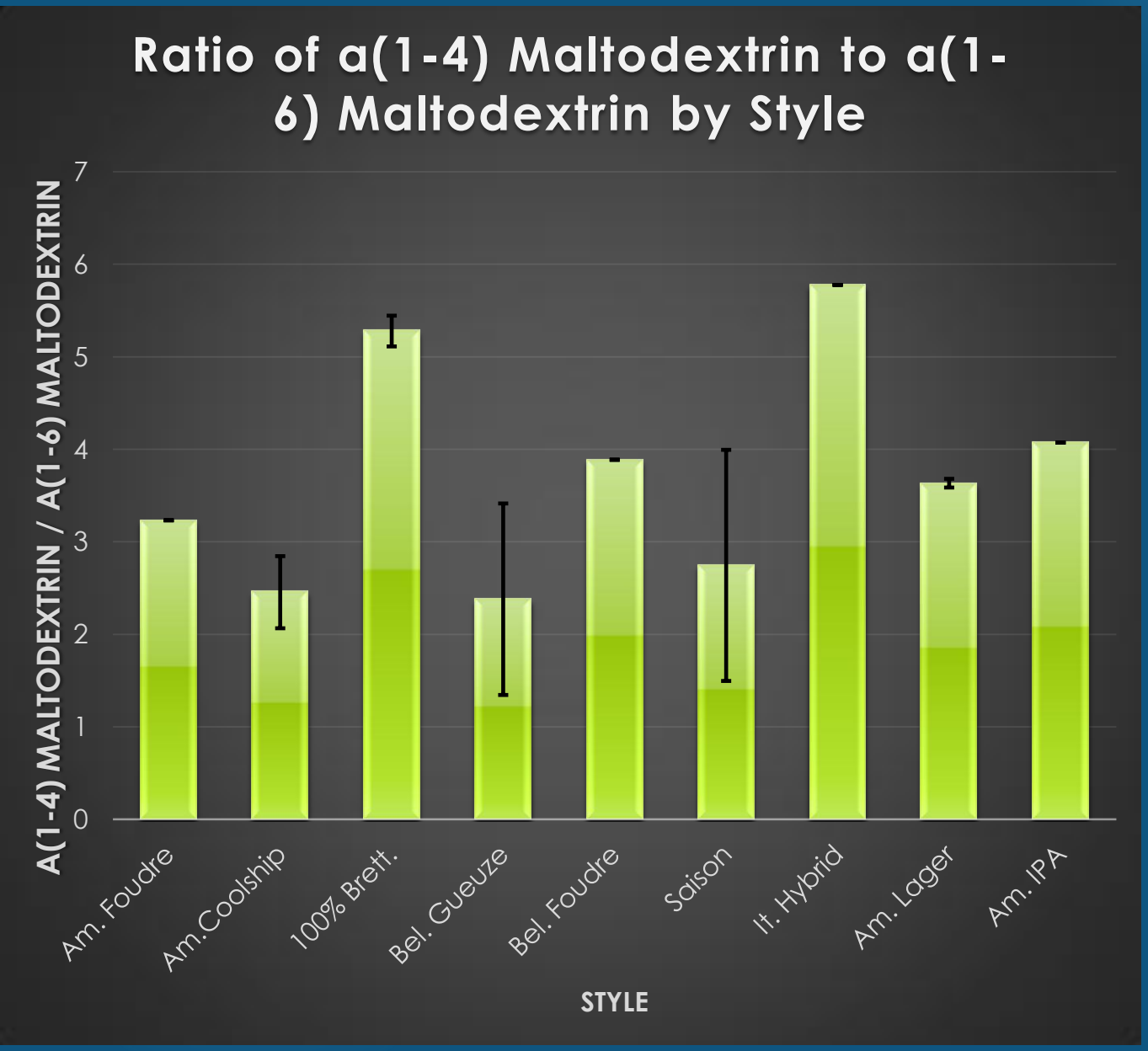


- Absolute concentrations of major components found in AWA (Acids: left y-axis, EtAc: right y-axis). The age of the beers refers to the fermentation time (in years) for the respective barrels sampled, as all samples came from separate batches. The 1 & 3 year old barrels were brewed in the Winter (November) and the 2 year old barrel was brewed in the Spring (May). Blend refers to the finished Gueuze-style AWA.



Anomeric ^{1}H region of the beer spectrum – maltose, dextrins and reduced sugars

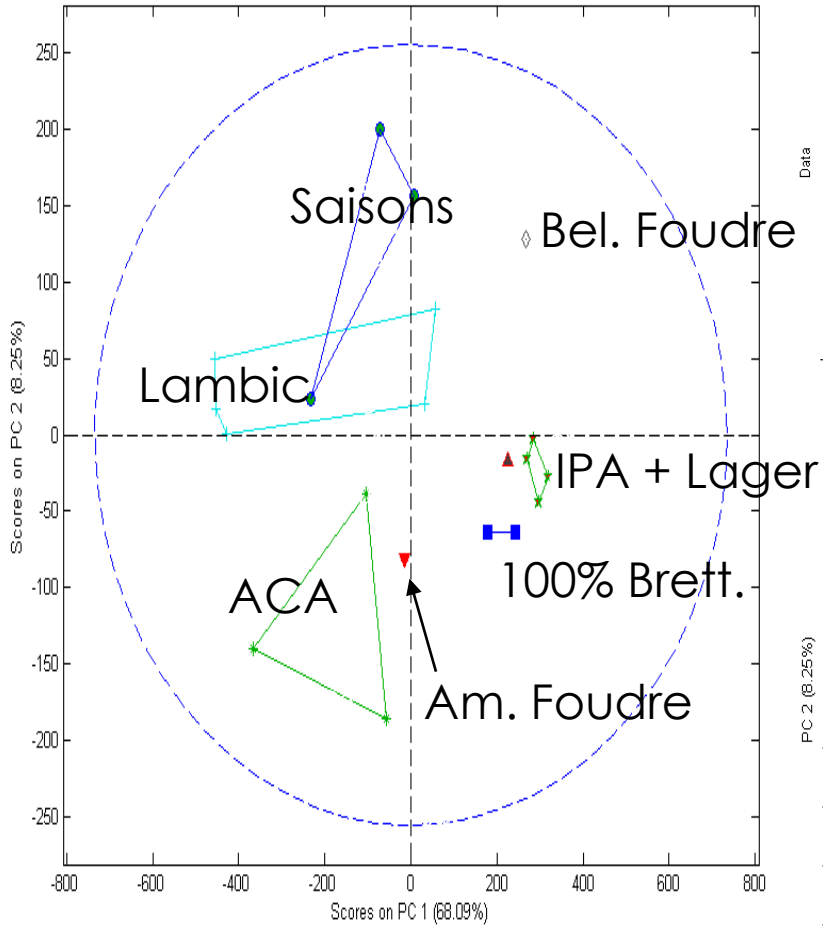
Dextrin Ratios

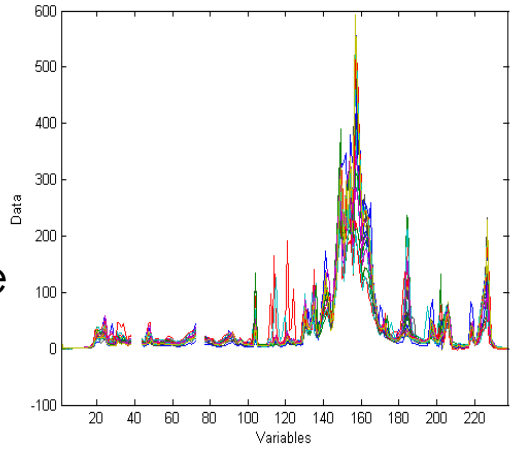


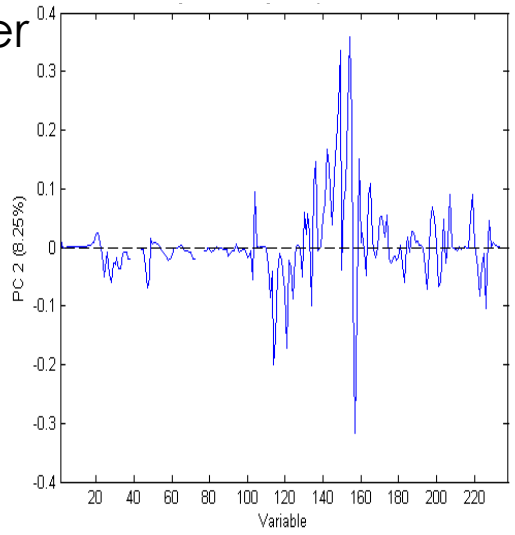
- "Degree of Fermentation"
  - Dependent on:
    - Strain
    - Style
    - Ingredients

Multivariate Analysis

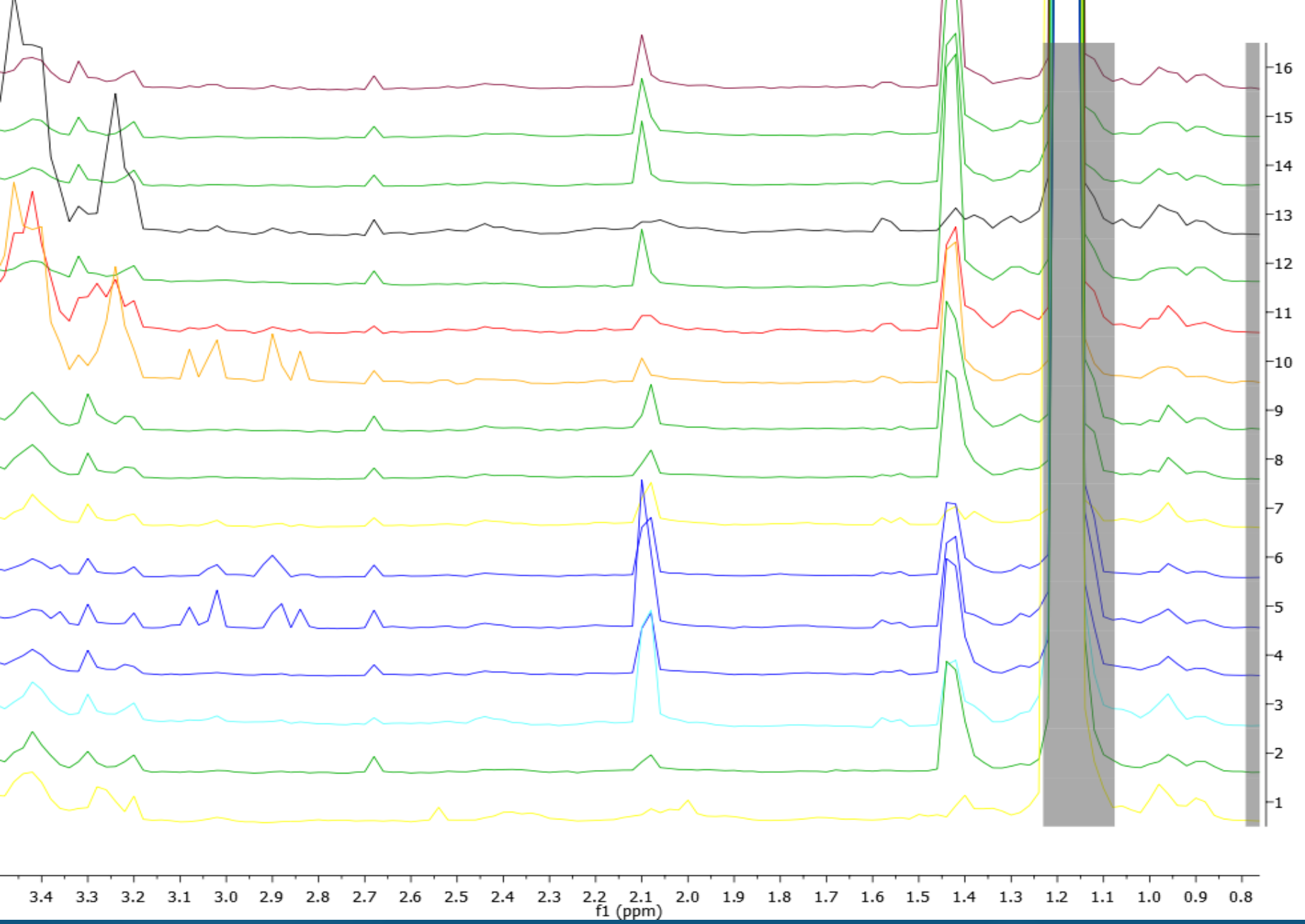
Principal Component Analysis







- Segregate based largely on sugar detail



PCA on Small Acid Region Only – Binned Data

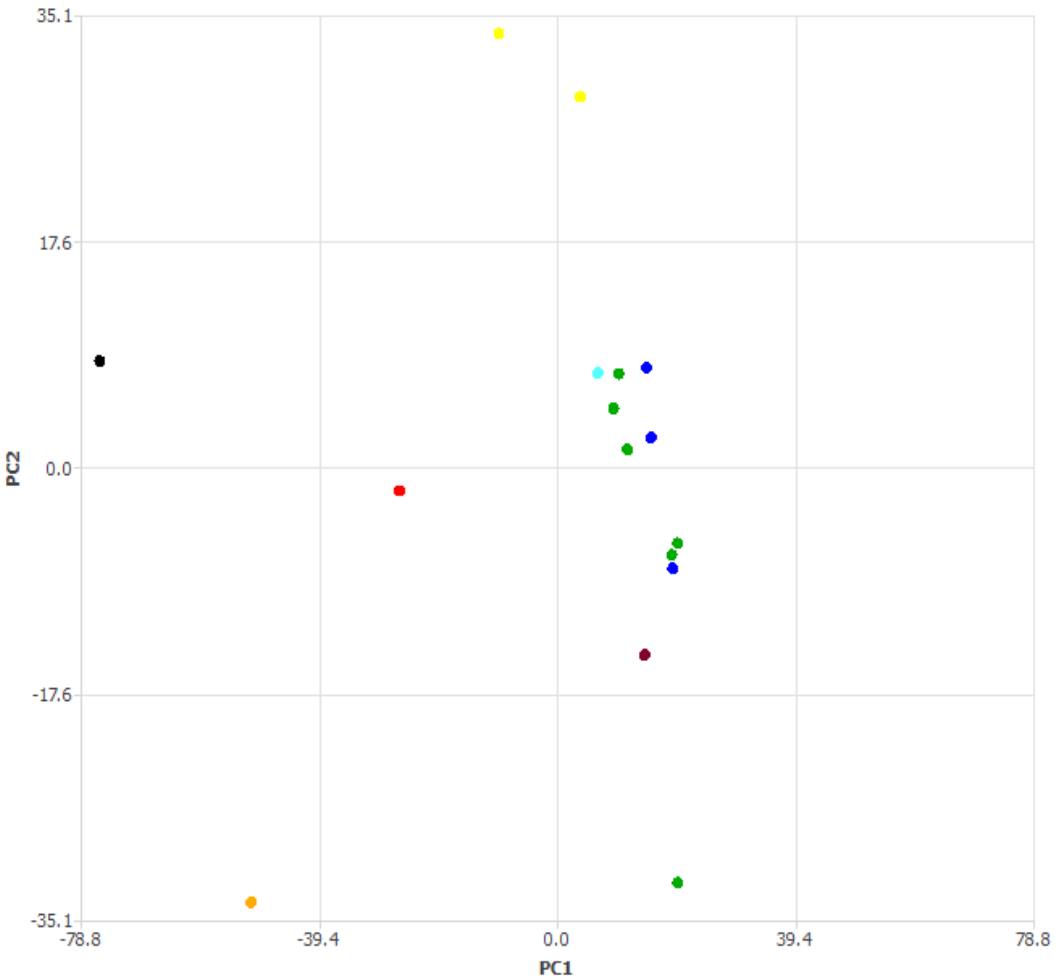
PCA Results

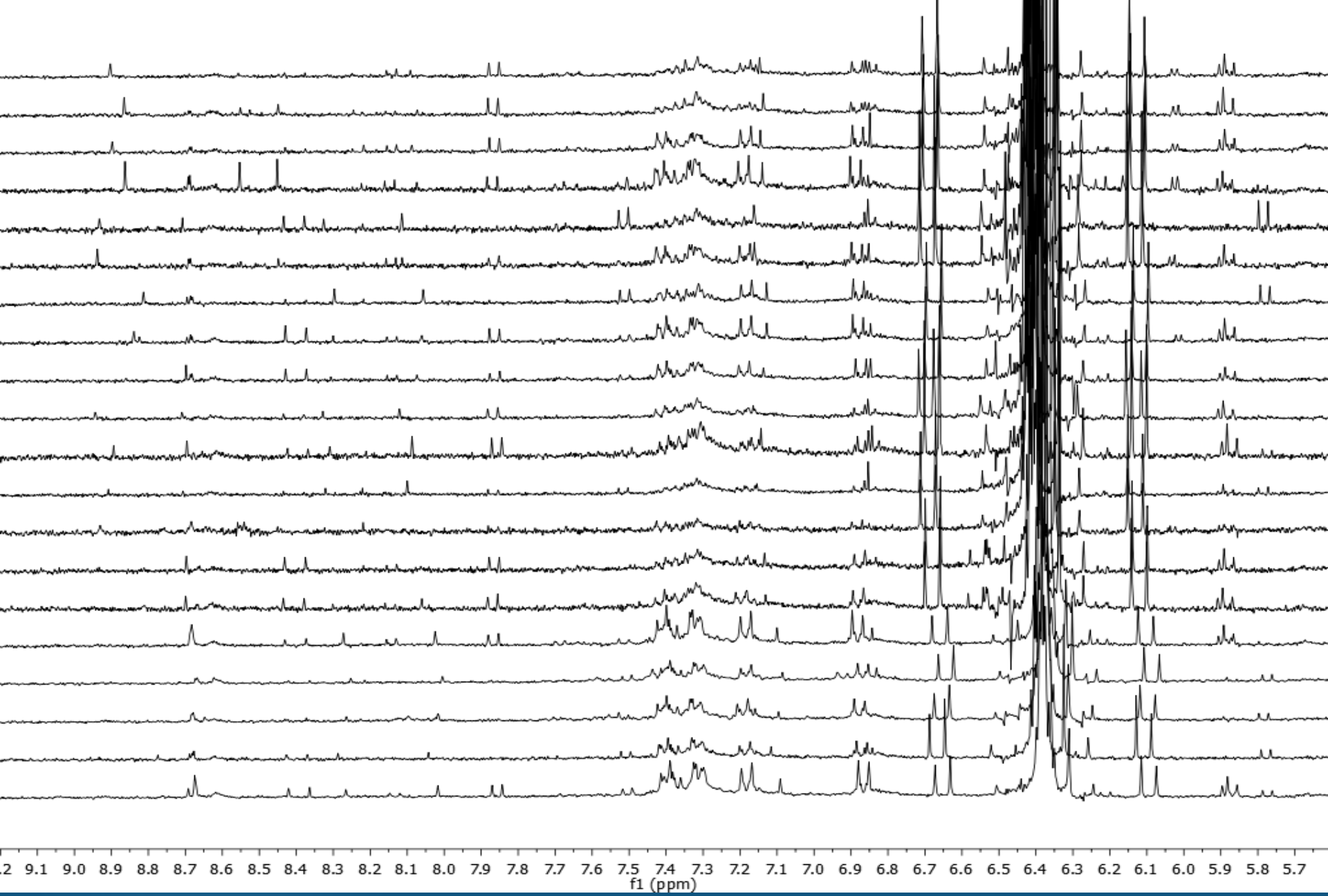
Results: Allagash-015-H
Drie Fonteinen Oude Gueuze II

Input | Principal Components | Score Plot | Loading Plot

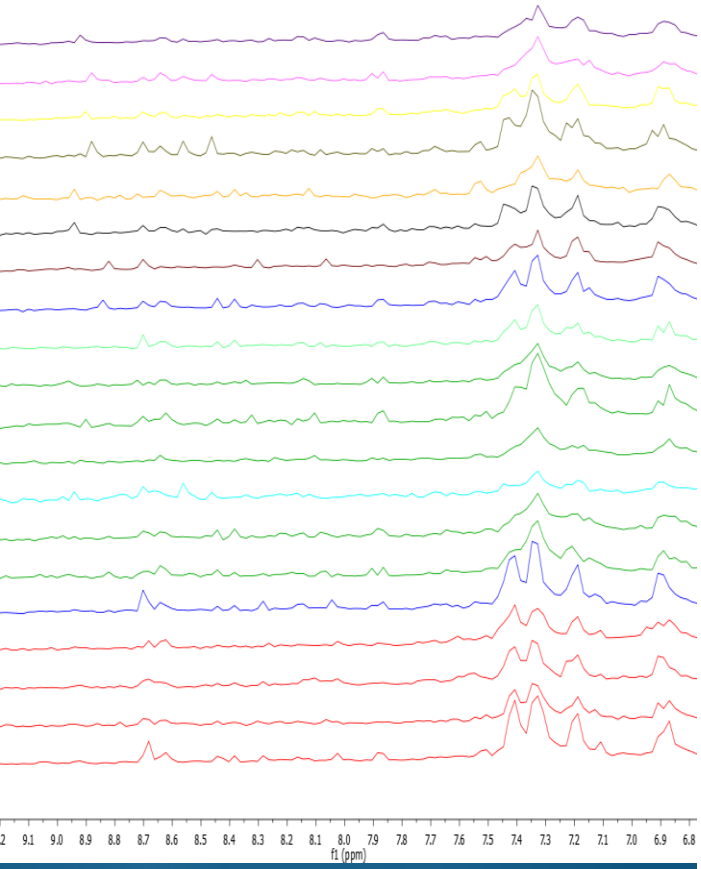
	Spectrum	Class	PC1	PC2
1	Allagash-001-H	Brett	-9.7938	33.7567
2	Allagash-002-H	Geuze	9.17306	4.67217
3	Allagash-003-H	Foudre	6.5788	7.40726
4	Allagash-004-H	Coolship	15.3622	2.41599
5	Allagash-005-H	Coolship	18.9564	-7.73856
6	Allagash-006-H	Coolship	14.5952	7.82633
7	Allagash-007-H	Brett	3.6744	28.8634
8	Allagash-008-H	Geuze	10.0229	7.36325
9	Allagash-009-H	Geuze	11.4422	1.49639
10	Allagash-010-H	Framboise	-50.6769	-33.6428
11	Allagash-011-H	Flemish Red	-26.1849	-1.71334
12	Allagash-012-H	Geuze	19.746	-32.1145
13	Allagash-013-H	Harvest Ale	-75.7216	8.3406
14	Allagash-014-H	Geuze	18.7648	-6.70183
15	Allagash-015-H	Geuze	19.7547	-5.78058
16	Allagash-016-H	Sour Saison	14.3065	-14.4505

PC1, PC2

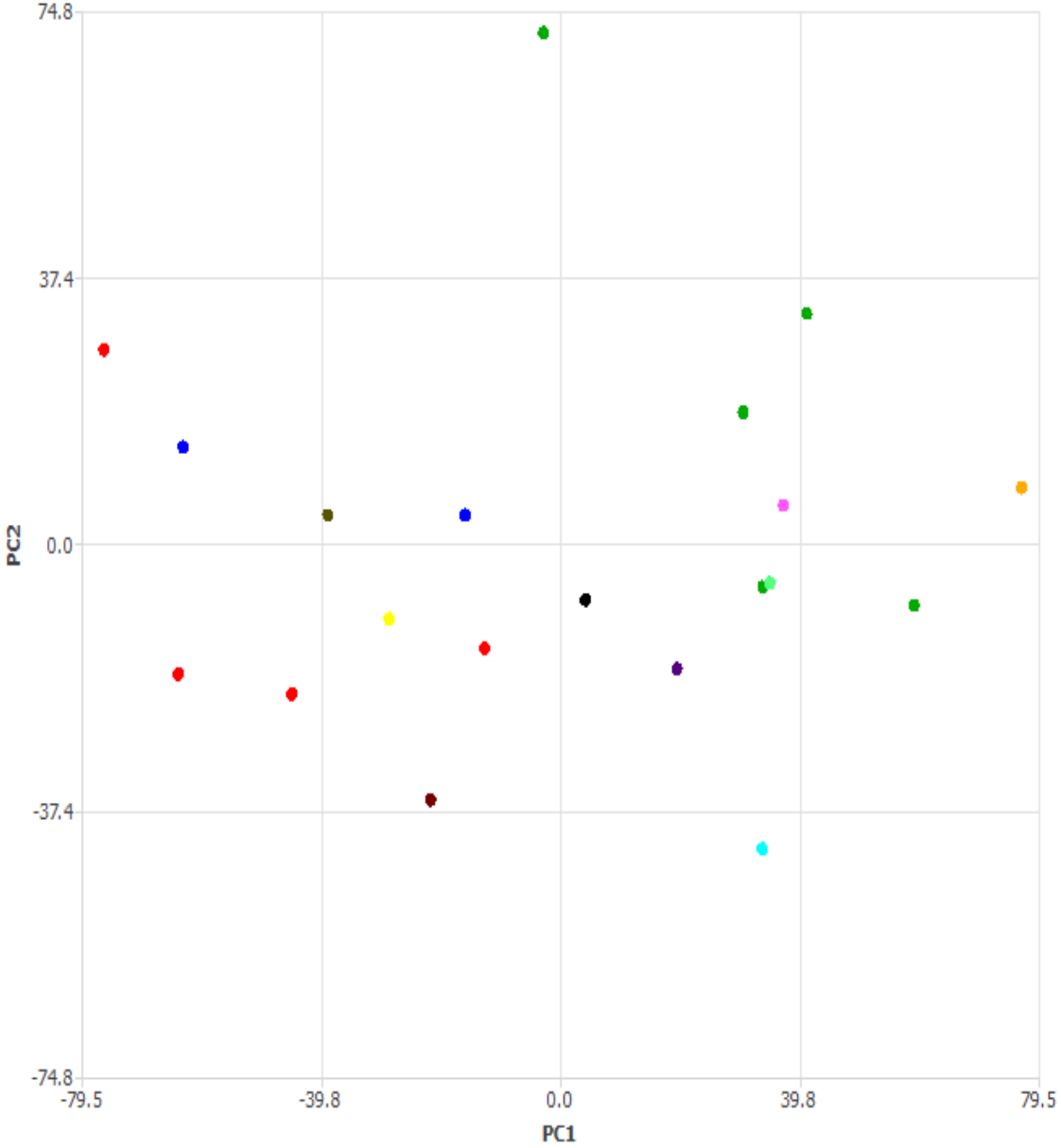




Aromatic Region of Beer Spectrum



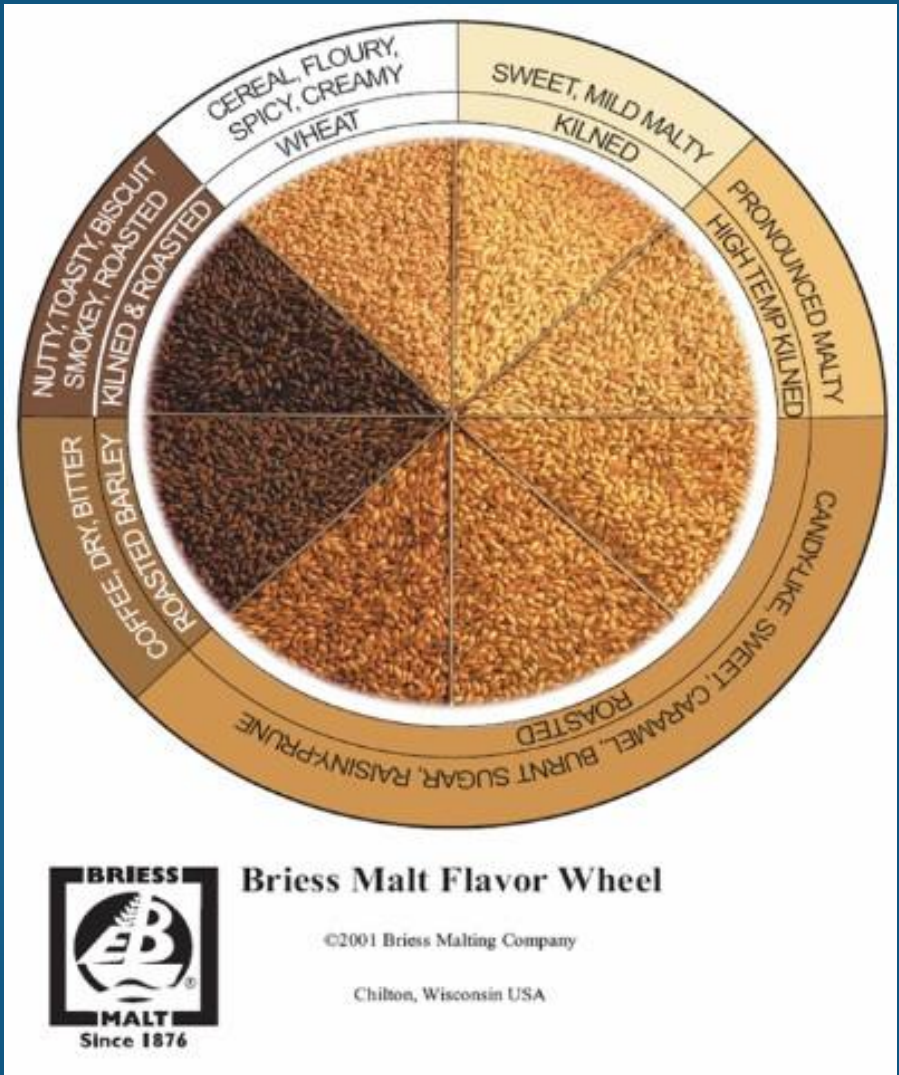
Poor Discrimination of Beer Styles from aromatic chemistry



Conclusions

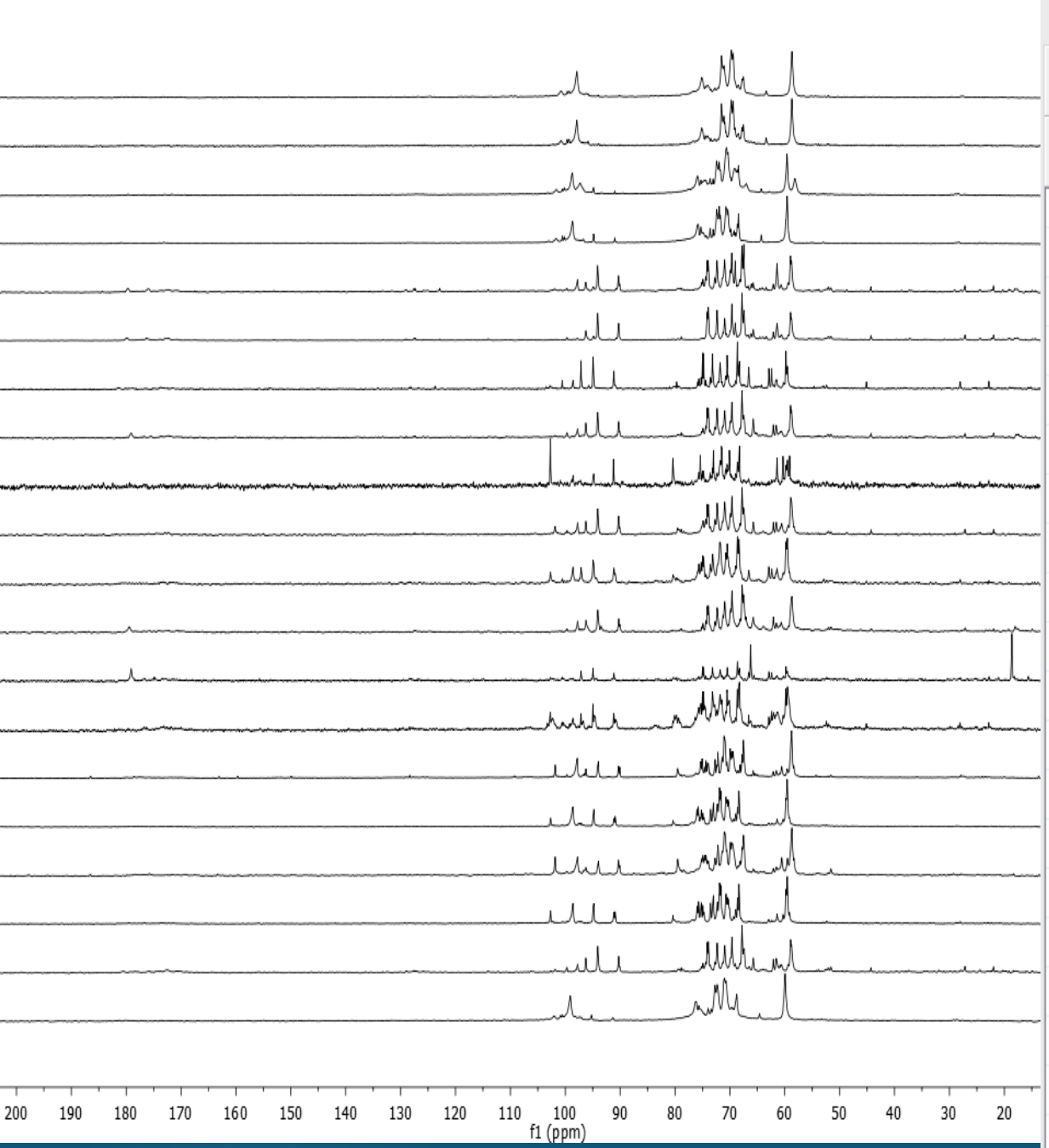
- ACAs & Belgian Lambics have differing acid profiles
- Ratio of linear and branched maltodextrins can be used to differentiate styles
- Multivariate analysis can differentiate between ACAs, Belgian Lambics and 6 other styles

Malt Chemistry



Malt	Lovibond
American Malts	
U.S. two-row	1.4 - 1 8
U.S. six-row	1.5 - 1.9
Pale Malt	2
Canadian two-row	1.3 - 1.7
Canadian six-row	1.4 - 1.9
Wheat Malt	2.2
Vienna Malt	4
Munich Malt	10
Carapils	2
Light Crystal	10
Pale Crystal	40
Medium Crystal	60
Dark Crystal	120
Victory Malt	25
Special Roast	50
Chocolate Malt	350
Roast Barley	300
Black Barley	450-500
Black Patent	500

Maillard Chemistry – Glucose + Amino Acid --→ Schiff Base



Stacked Spectra

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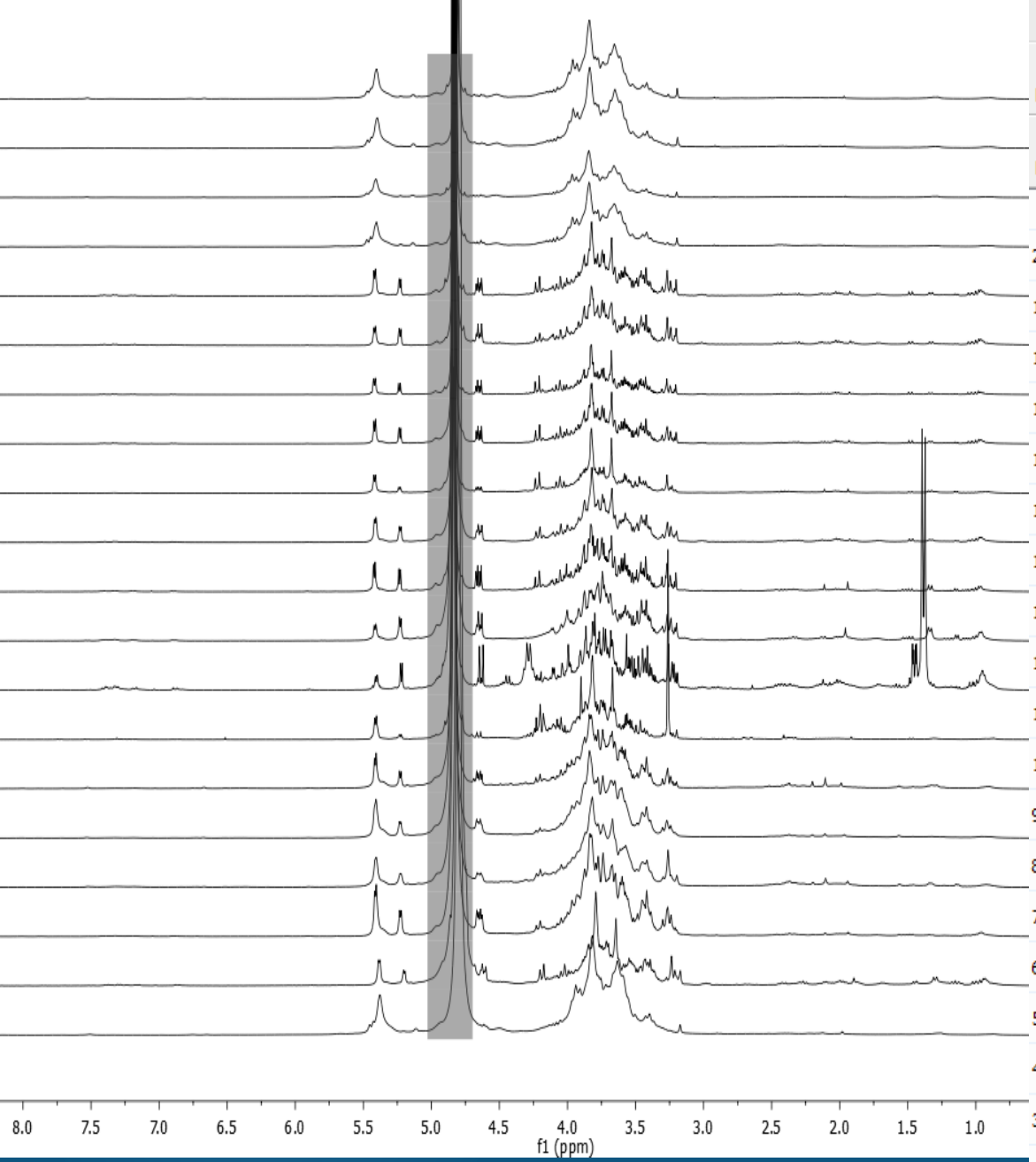
		Title
20	☑	Malt-021-C_FDMuntons Black Patent 515oL
19	☑	**Malt-020-C_FDWeyermann Unmalted Roasted Barley 435oL**
18	☑	Malt-019-C_FDMuntons Chocolate Malt 395oL
17	☑	Malt-018-C_FDBriess Midnight Wheat 550oL
16	☑	Malt-017-C_FDMuntons Pale Malt 2.53oL
15	☑	Malt-016-C_FDCanadian Pale 2-Row 1.9oL
14	☑	Malt-015-C_FDWeyermann Vienna Malt 3oL
13	☑	Malt-014-C_FDWeyermann 5.65oL
12	☑	Malt-013-C_FDBriess Victory Malt
11	☑	Malt-012-C_FDWayermann Smoked Malt 2.75oL
10	☑	Malt-011-C_FDDingeman's Aromatic Malt 20oL
9	☑	Malt-010-C_FDGambrinus Honey Malt 17.5oL
8	☑	Malt-009-C_FDWeyermanns Acidulated Malt 2.25oL
7	☑	Malt-008-C_FDWeyermann Rye Malt 3.55oL
6	☑	Malt-006-C_FDDingemans Special B 118oL
5	☑	Malt-005-C_FDBriess Caramel Malt 6-Row 80oL
4	☑	Malt-004-C_FDBriess Caracrystal Wheat 45oL
3	☑	Malt-003-C_FDBriess Caramel Malt 6-Row 20oL
2	☑	Malt-002-CWeyermann Carafoam 2-Row Barley 1.7-2.4oL
1	☑	Malt-001-C_FDDingemans Debittered Black Patent 509oL

^{13}C NMR – Malt/Hot Water Extracts



^{1}H NMR – Malt/Hot Water Extracts

Stacked Spectra

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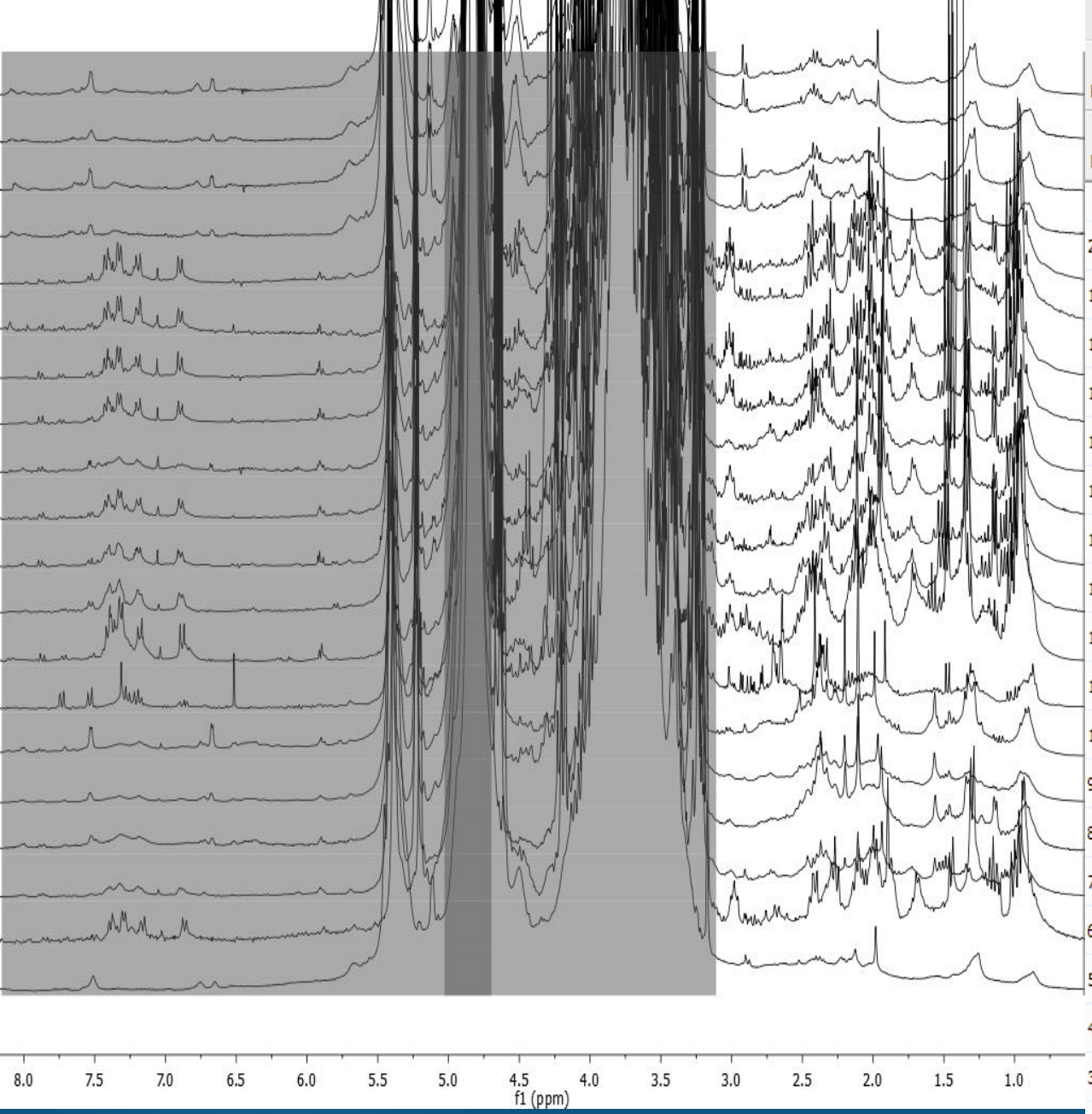
	Title
20	Malt-021-H_FDMunton's Black Patent 515oL
19	Malt-020-H_FDWeyermann Unmalted Roasted Barley 435oL
18	Malt-019-H_FDMunton's Chocolate Malt 395oL
17	Malt-018-H_FDBriess Midnight Wheat 550oL
16	Malt-017-H_FDMunton's Pale Malt 2.53oL
15	Malt-016-H_FDanadian Pale 2-Row 1.9oL
14	Malt-015-H_FDWeyermann Vienna 3oL
13	Malt-014-H_FDWeyermann Light Munich 5.65oL
12	Malt-013-H_FDBriess Victory Malt 25oL
11	Malt-012-H_FDWeyermann Smoked Malt 2.75oL
10	**Malt-011-H_FDDingeman's Aromatic Malt 20oL**
9	Malt-010-H_FDGambrinus Honey Malt 17.5oL
8	Malt-009-H_FDWeyermann Acidulated Malt 2.25oL
7	Malt-007-H_FDMuntons Torrified Wheat
6	Malt-006-H_FDDingemans Special B 118oL
5	Malt-005-H_FDBriess Caramel Malt 6-Row Barley 80oL
4	Malt-004-H_FDBriess Caracrystal Wheat 45oL
3	Malt-003-H_FDBriess Caramel Malt 6-Row 20oL
2	Malt-002-HWeyermann Carafoam ~2.05L
1	Malt-001-HDingeman's Black Patent Debittered Moot Roast 509L



Stacked Spectra

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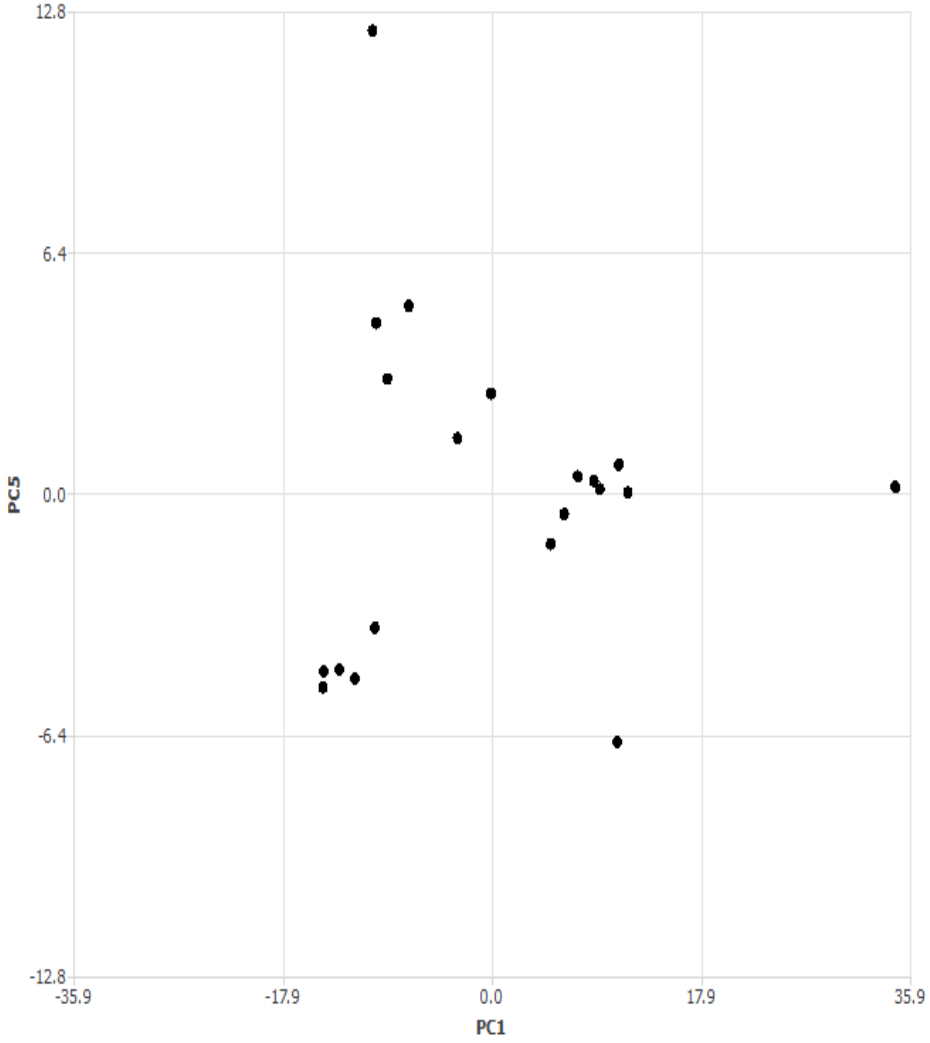
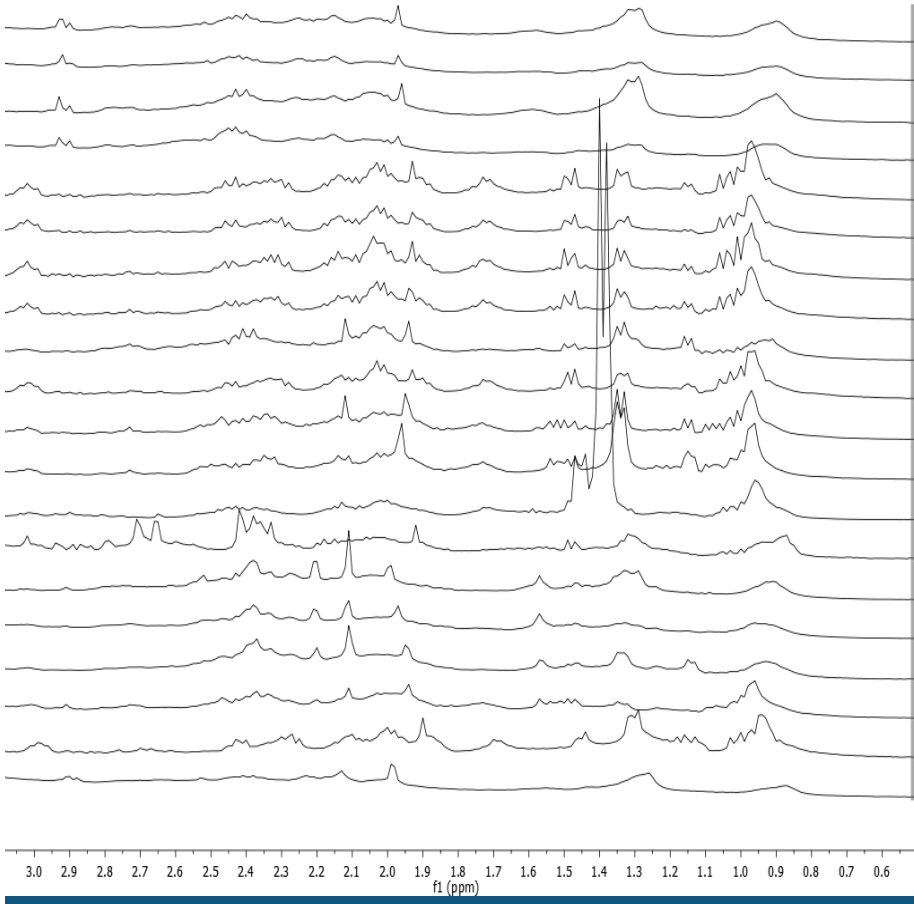
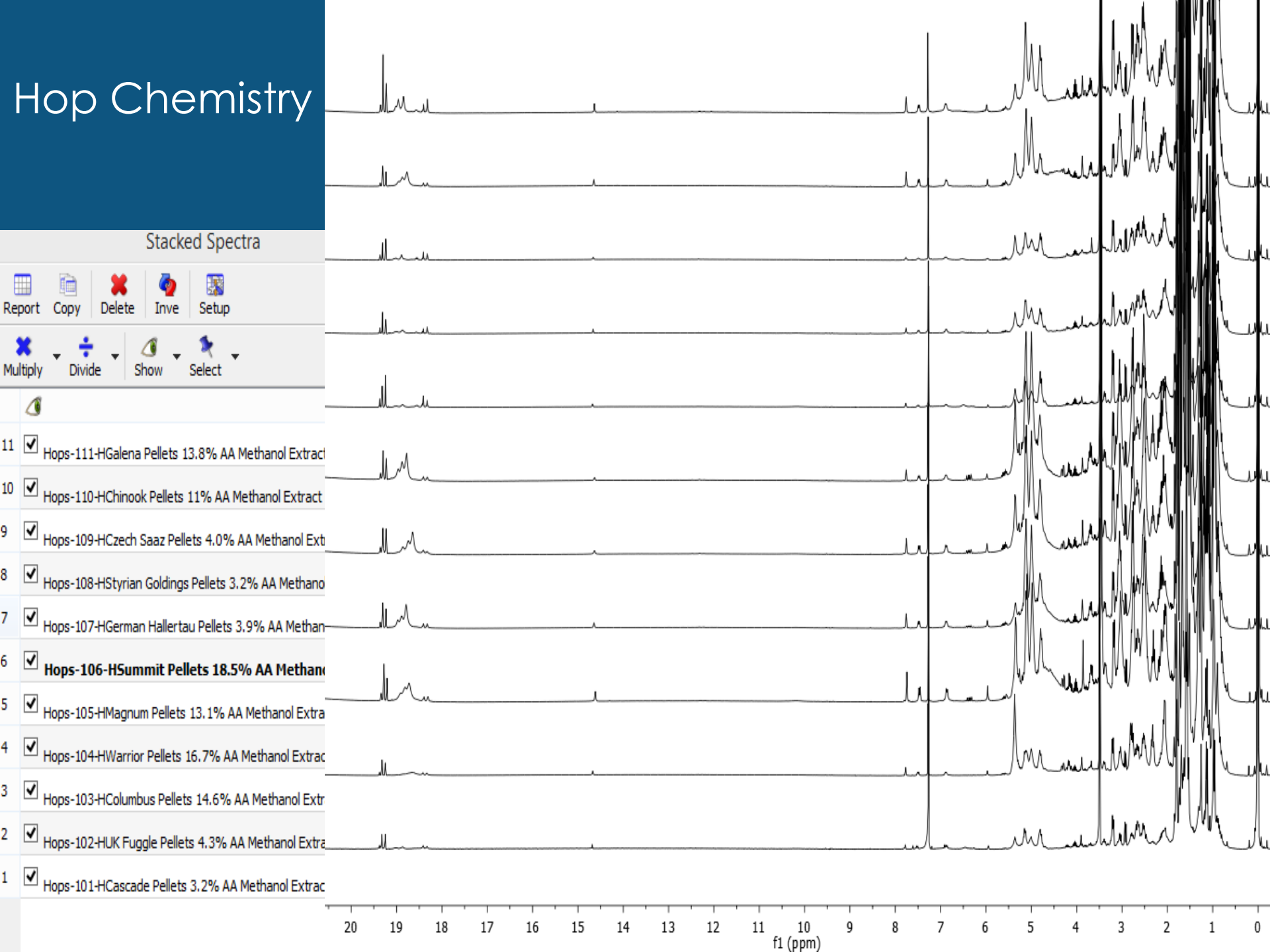
	Title
20	Malt-021-H_FDMunton's Black Patent 515oL
19	Malt-020-H_FDWeyermann Unmalted Roasted Barley 435oL
18	Malt-019-H_FDMunton's Chocolate Malt 395oL
17	Malt-018-H_FDBriess Midnight Wheat 550oL
16	Malt-017-H_FDMunton's Pale Malt 2.53oL
15	Malt-016-H_FDanadian Pale 2-Row 1.9oL
14	Malt-015-H_FDWeyermann Vienna 3oL
13	Malt-014-H_FDWeyermann Light Munich 5.65oL
12	Malt-013-H_FDBriess Victory Malt 25oL
11	Malt-012-H_FDWeyermann Smoked Malt 2.75oL
10	**Malt-011-H_FDDingeman's Aromatic Malt 20oL**
9	Malt-010-H_FDGambrinus Honey Malt 17.5oL
8	Malt-009-H_FDWeyermann Acidulated Malt 2.25oL
7	Malt-007-H_FDMuntons Torrified Wheat
6	Malt-006-H_FDDingemans Special B 118oL
5	Malt-005-H_FDBriess Caramel Malt 6-Row Barley 80oL
4	Malt-004-H_FDBriess Caracrystal Wheat 45oL
3	Malt-003-H_FDBriess Caramel Malt 6-Row 20oL
2	Malt-002-HWeyermann Carafoam ~2.05L
1	Malt-001-HDingeman's Black Patent Debittered Moot Roast 509L

Multivariate Analysis and Chemistry Identification





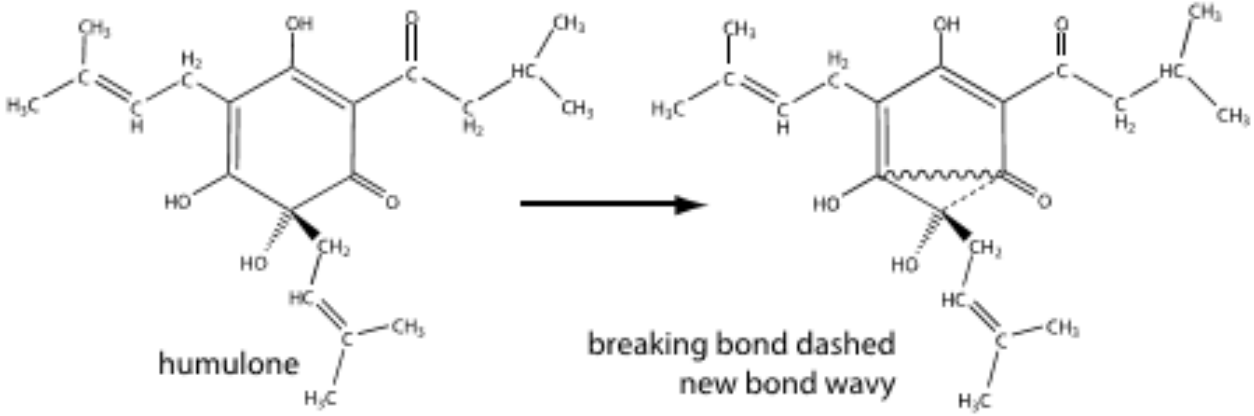
Hop Chemistry



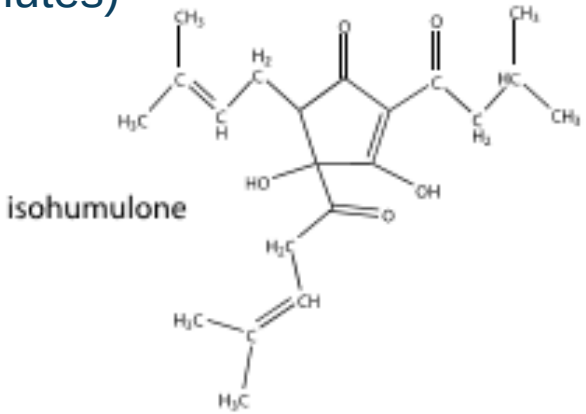
Hops – Bittering, Flavor, Aroma, Foam Stability

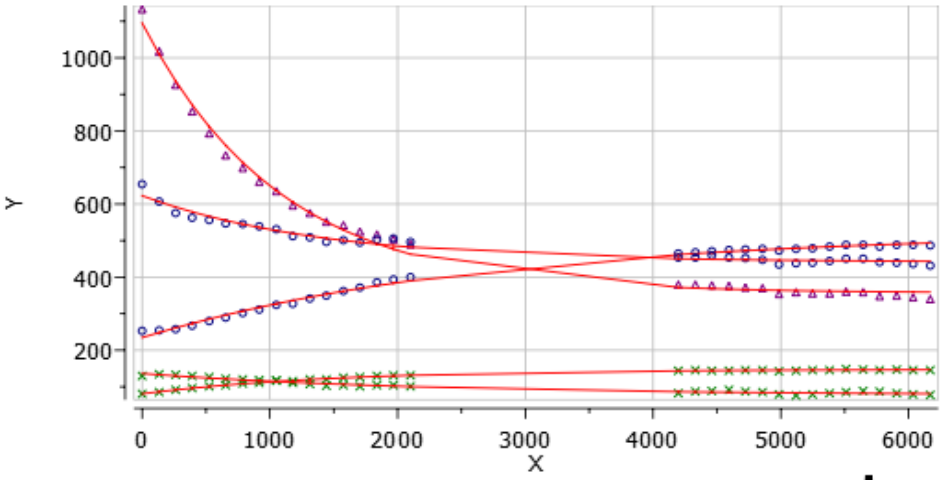
Hops in beer – are the acids and essential oils of

Alpha acids (humulone, adhumulone, cohumulone)
Beta acids (primarily **humulene**, **myrcene**, **caryophyllene** and **farnesene**)



Alpha acids isomerization to iso-alpha acids over the course of boiling the beer wort (60+ minutes)





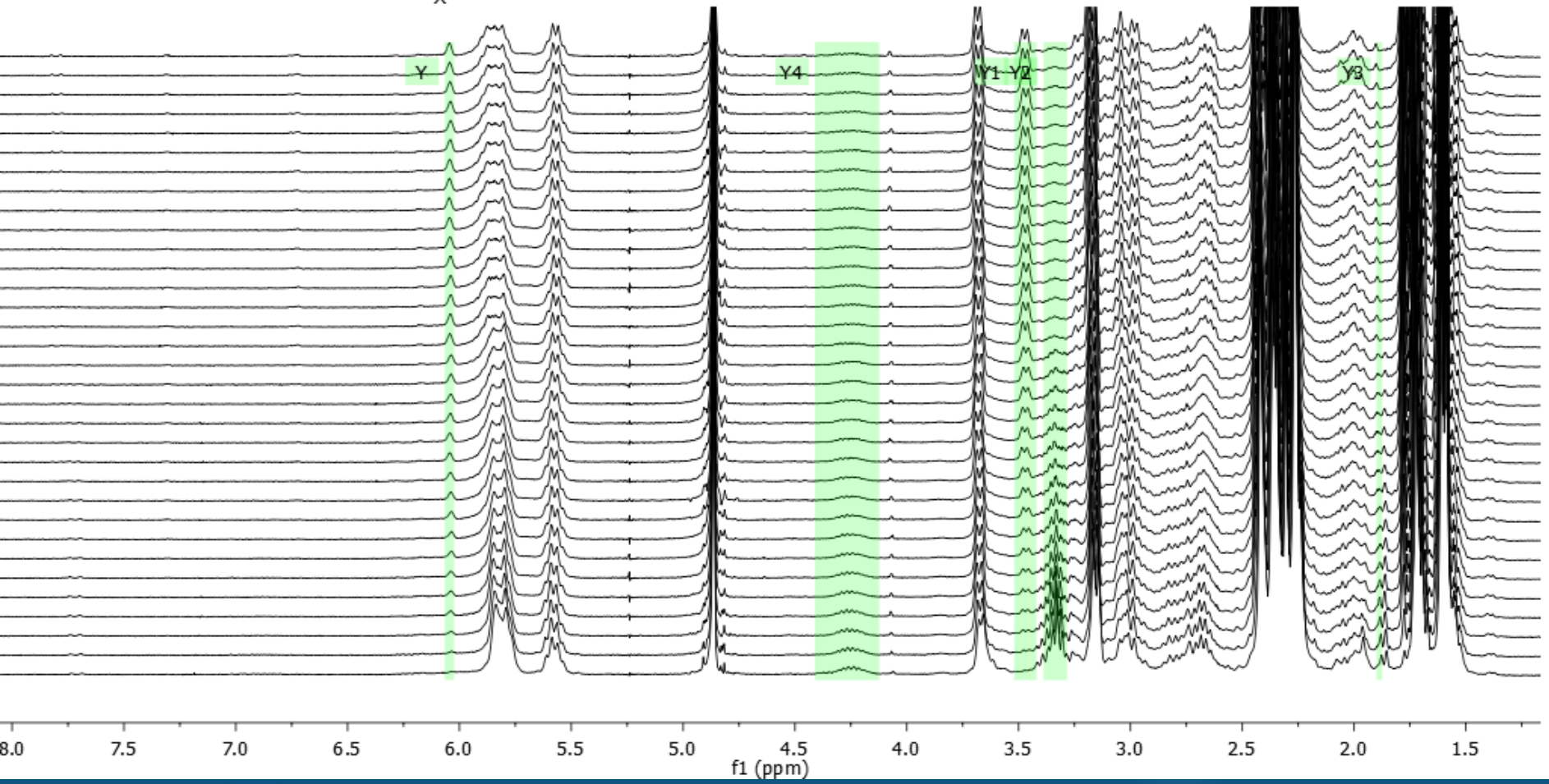
Y' = B+F*exp(-X*G)
B=147.5; F=-66.93; G=0.0006707;
Error: 0.00001121

Y1' = B+F*exp(-X*G)
B=522.9; F=-288.5; G=0.0003683;
Error: 0.00001375

Y2' = B+F*exp(-X*G)
B=356.4; F=739.0; G=0.0009233;
Error: 0.00001882

Y3' = B+F*exp(-X*G)
B=77.14; F=59.05; G=0.0004443;
Error: 0.00004140

Y4' = B+F*exp(-X*G)
B=441.1; F=181.5; G=0.0007098;
Error: 0.00004602

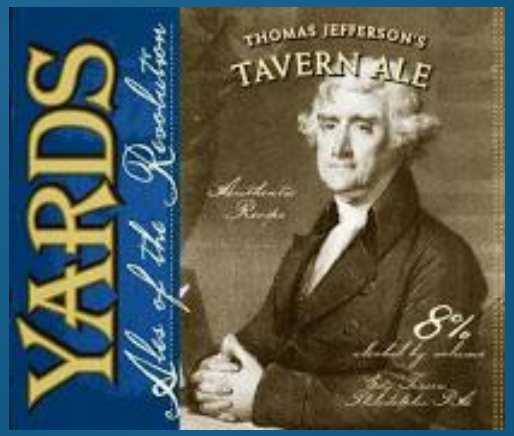


More Ongoing Fermentation Projects

- Yards Brewing – Complete analysis of the brewing process from mash through finished bottled products – Thomas Jefferson Ale
- NMR and Portable MS analysis of beer production process at Mill Street Brewery, Poughkeepsie
- Expand to include Mead and Cider

Acknowledgments

Adam DiCaprio – needs a job in the Raleigh-Durham Area



Yards Brewing Company, Philadelphia PA

Allagash Brewing Company, Portland ME