

# Small Molecule Chemistry of Spontaneously Fermented Coolship Ales

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## Background

Spontaneously fermented beers have been an integral part of the Belgian brewing tradition for many hundreds of years, and have given rise to a diverse family of "lambic" beers, including geuze, kriek, fardo, flanders ales and fruit lambics. These beers all share the characteristic dry acidity derived from the "spontaneously" derived fermentative organisms involved in the production of these ales.

Production of many of these beers involves the overnight cooling of unfermented beer (wort) in large, open coolships and the eventual transfer to wood aging vessels. During this time the wort is organically inoculated with wild microbes including *Saccharomyces spp.*, *Brettanomyces spp.*, *Acetobacter spp.* and many members of the *Lactobacillaceae*, all totaling more than 70 different species<sup>1</sup>. Over the course of months to years, these wild microbes are responsible for the production of numerous small flavor-active compounds, all of which contribute to the complex nature of these beers<sup>2,3</sup>.



Figure 1: Coolship at Brasserie Cantillon Brouwerij. Photo Credit: Jim Rush



Figure 2: Spontaneously fermenting beer at Brewery Timmermans

Recently, the explosive diversification and growth of the craft brewing industry in the United States has led to the creation and expansion of the "American Wild Ale" style, which include beers brewed in a manner nearly identical to that of the traditional Belgian lambic, excepting geographical differences. In this project, quantitative <sup>1</sup>H-NMR methodology<sup>4</sup> and multivariate discriminate analysis was used to investigate and quantify the key "macro-chemistry" of typical American Wild Ales in relation to commonly found Belgian lambic.

## Methods

- Beer was purchased at a local distributor
- Three American Coolship Ales were acquired directly from a small, commercial production brewery in the United States
- For straight runs 175µL de-gassed beer was added to 575µL D<sub>2</sub>O and a known mass of maleic acid standard
- For freeze dried runs 500µL de-gassed beer was added to a massed vial and dried on a manifold freeze drier. Dried samples were re-hydrated in 750µL D<sub>2</sub>O + a known mass of maleic acid
- Samples were run on a Varian Mercury 300 spectrometer operating at 299.942 MHz, with a recycle delay of 5 seconds and an acquisition time of 7 seconds: 256 transients were collected for straight runs & 128 transients were collected for freeze dried runs
- Spectra were imported and processed in ACD/Labs and Mnova. Chemometric modeling was performed using Eigenvector Solo+Model\_Exporter
- Quantitation was performed using the following formula:

$$C_x = \frac{I_x}{I_{st}} \cdot \frac{N_{st}}{N_x}$$

Where c = Molar concentration, I = signal Integral & N = Hydrogens represented by "1" (subscript "x" represents target analyte and subscript "st" represents the internal standard)

## Spectra and Assignments

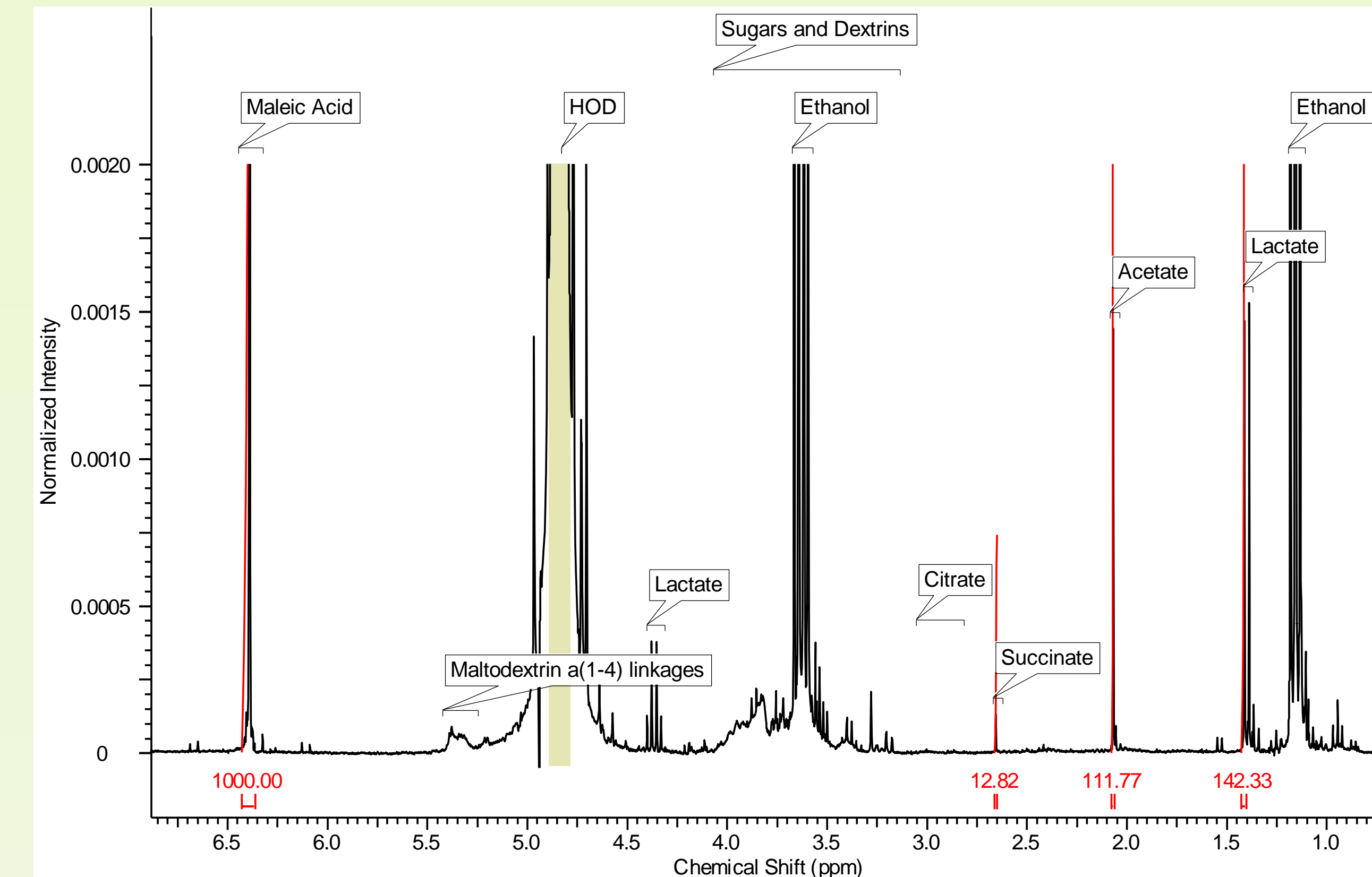


Figure 3: Straight run sample of an American Geuze-style beer with basic assignments. Integrals indicated are for quantified species

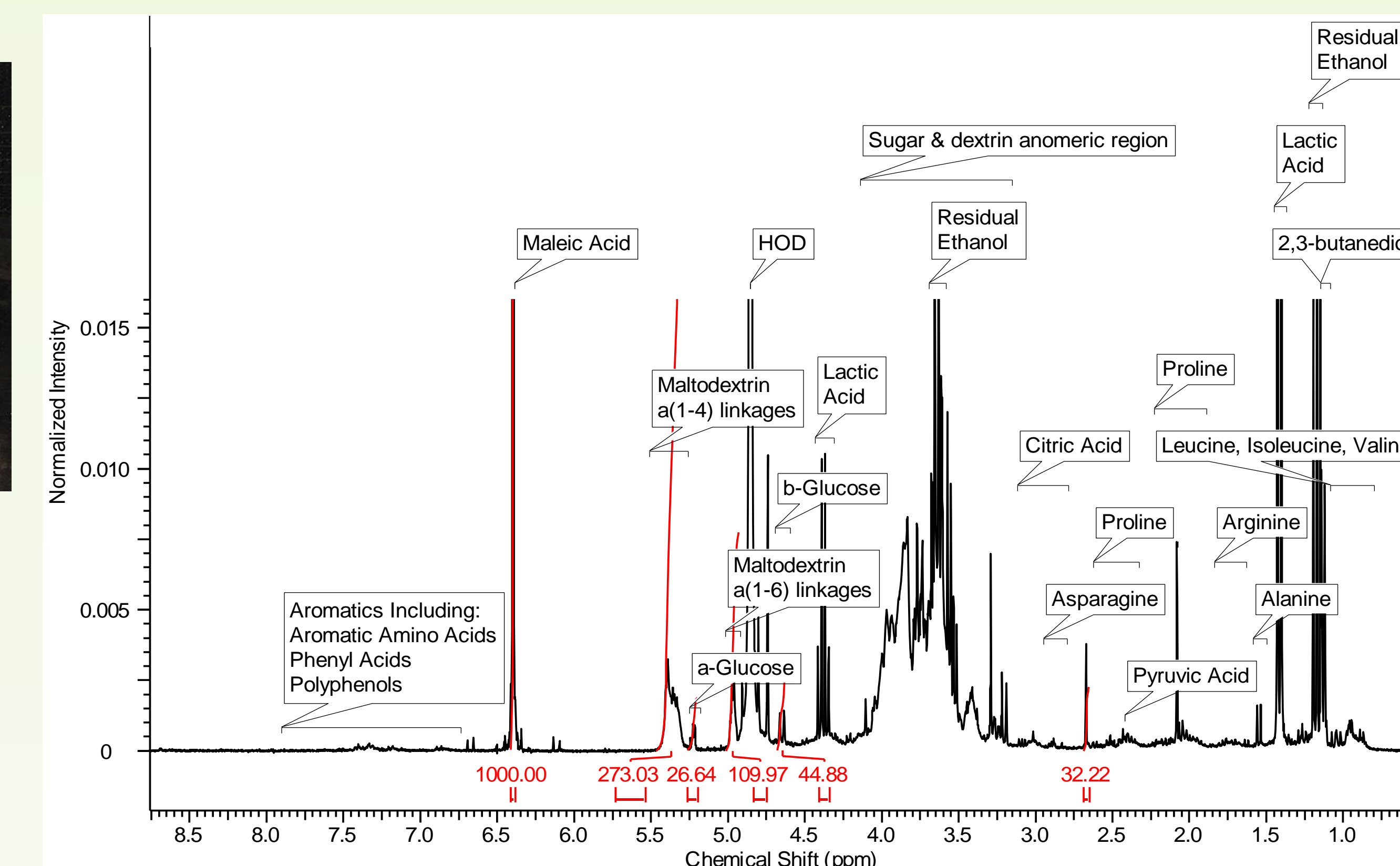


Figure 4: Freeze dried sample of an American Geuze-style beer with assignments. Integrals indicate quantified species.

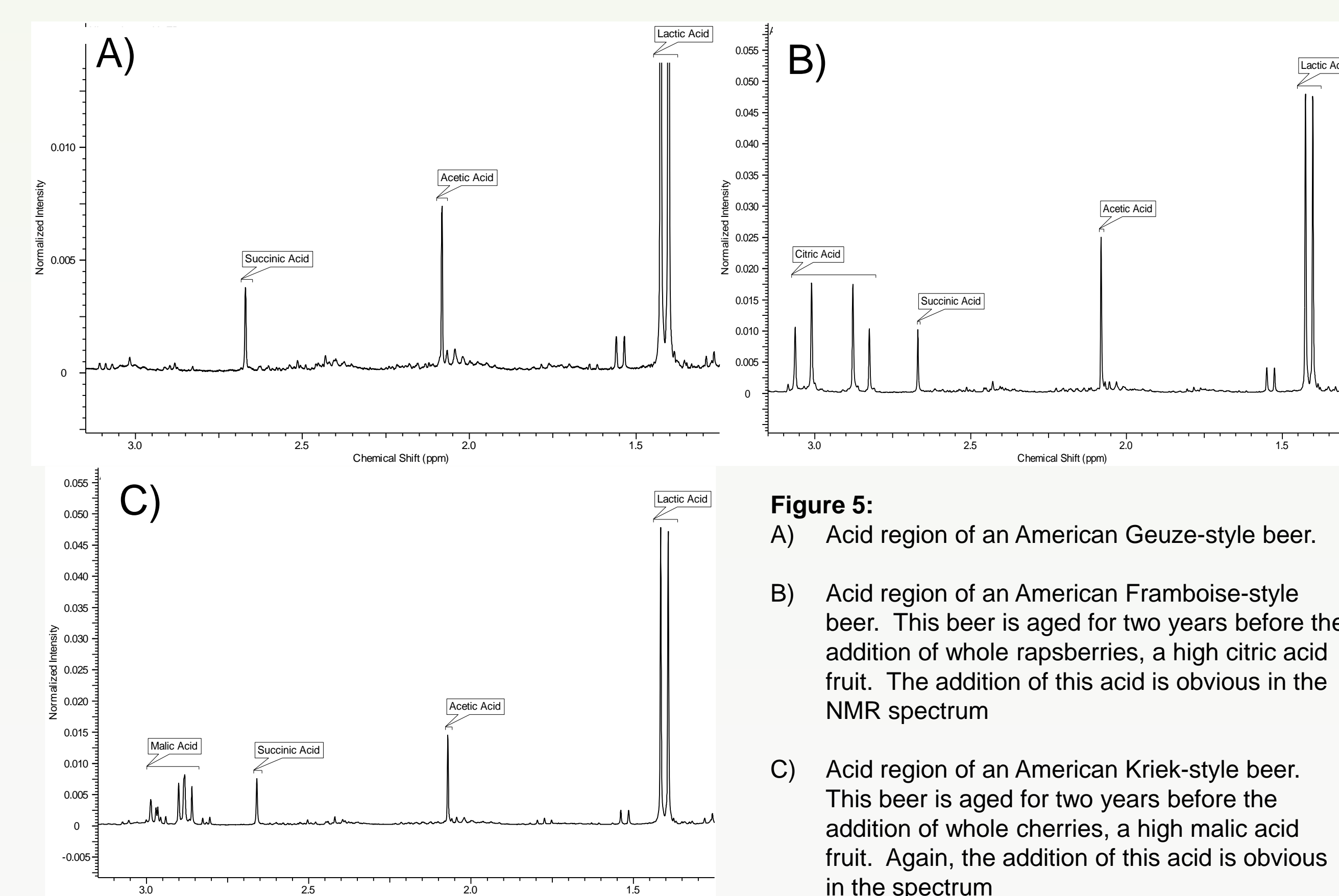


Figure 5:  
A) Acid region of an American Geuze-style beer.  
B) Acid region of an American Framboise-style beer. This beer is aged for two years before the addition of whole raspberries, a high citric acid fruit. The addition of this acid is obvious in the NMR spectrum  
C) Acid region of an American Kriek-style beer. This beer is aged for two years before the addition of whole cherries, a high malic acid fruit. Again, the addition of this acid is obvious in the spectrum

## Results

Table 1: Absolute value concentrations of target organic acids in spontaneously fermented beers. Quantitation of organic acids was performed on "straight-run" samples using maleic acid as an internal standard and measured sample/solvent volumes to calculate absolute concentrations by manual peak integration. LA = Lactic Acid, AA = Acetic Acid, SA = Succinic Acid, CA = Citric Acid, MA = Malic Acid.

| 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                    | 4506.5    | 488.1     | 217.0     | 0         | 0         |
| Parfait 2009             | 3497.8    | 454.1     | 175.2     | 0         | 0         |
| Geuze Fond Tradition     | 6807.8    | 698.6     | 218.8     | 0         | 0         |
| Drie Fontein A           | 5137.6    | 865.9     | 234.6     | 0         | 0         |
| Drie Fontein B           | 5389.9    | 917.7     | 228.9     | 0         | 0         |

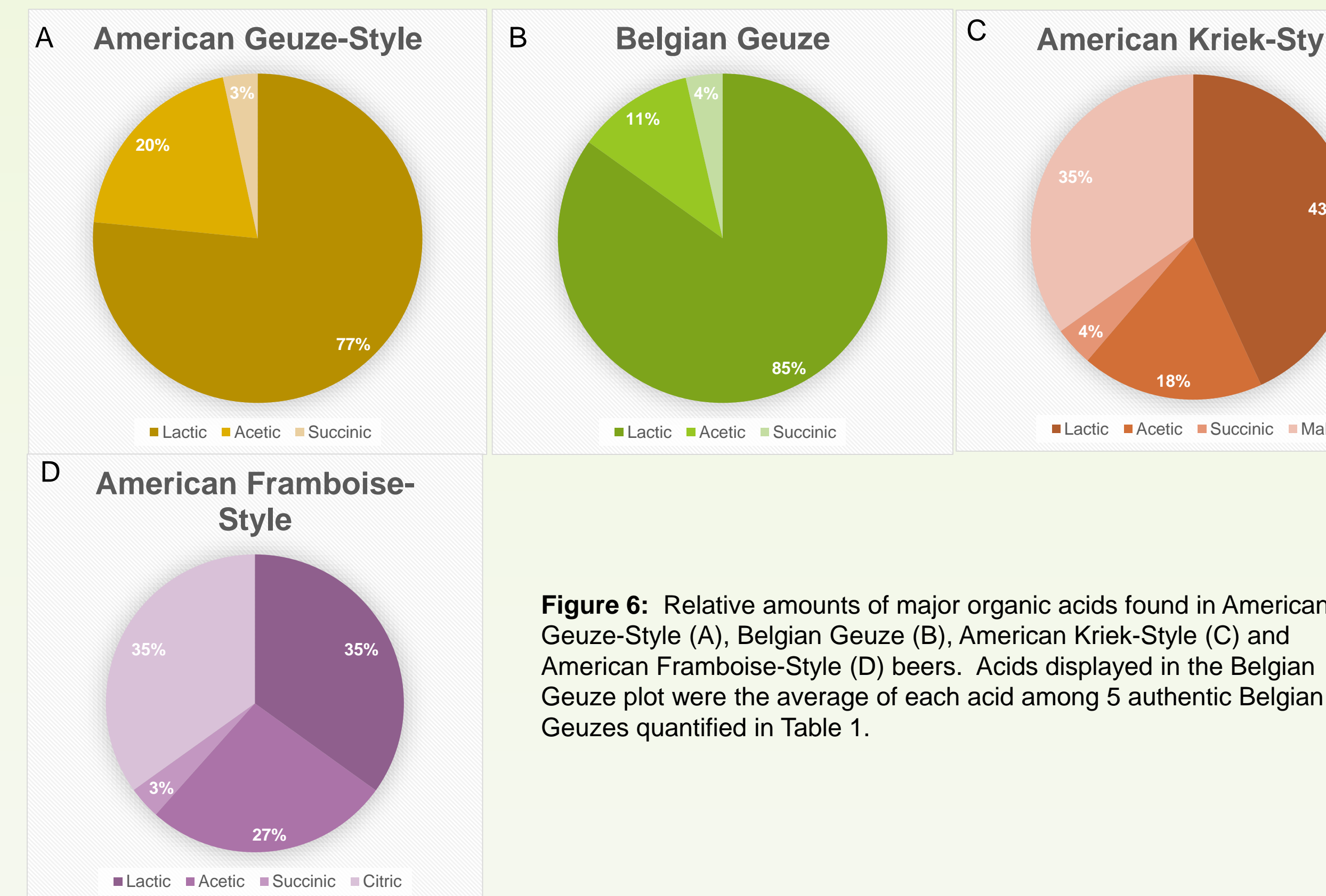


Figure 6: Relative amounts of major organic acids found in American Geuze-Style (A), Belgian Geuze (B), American Kriek-Style (C) and American Framboise-Style (D) beers. Acids displayed in the Belgian Geuze plot were the average of each acid among 5 authentic Belgian Geuzes quantified in Table 1.

## Results Continued

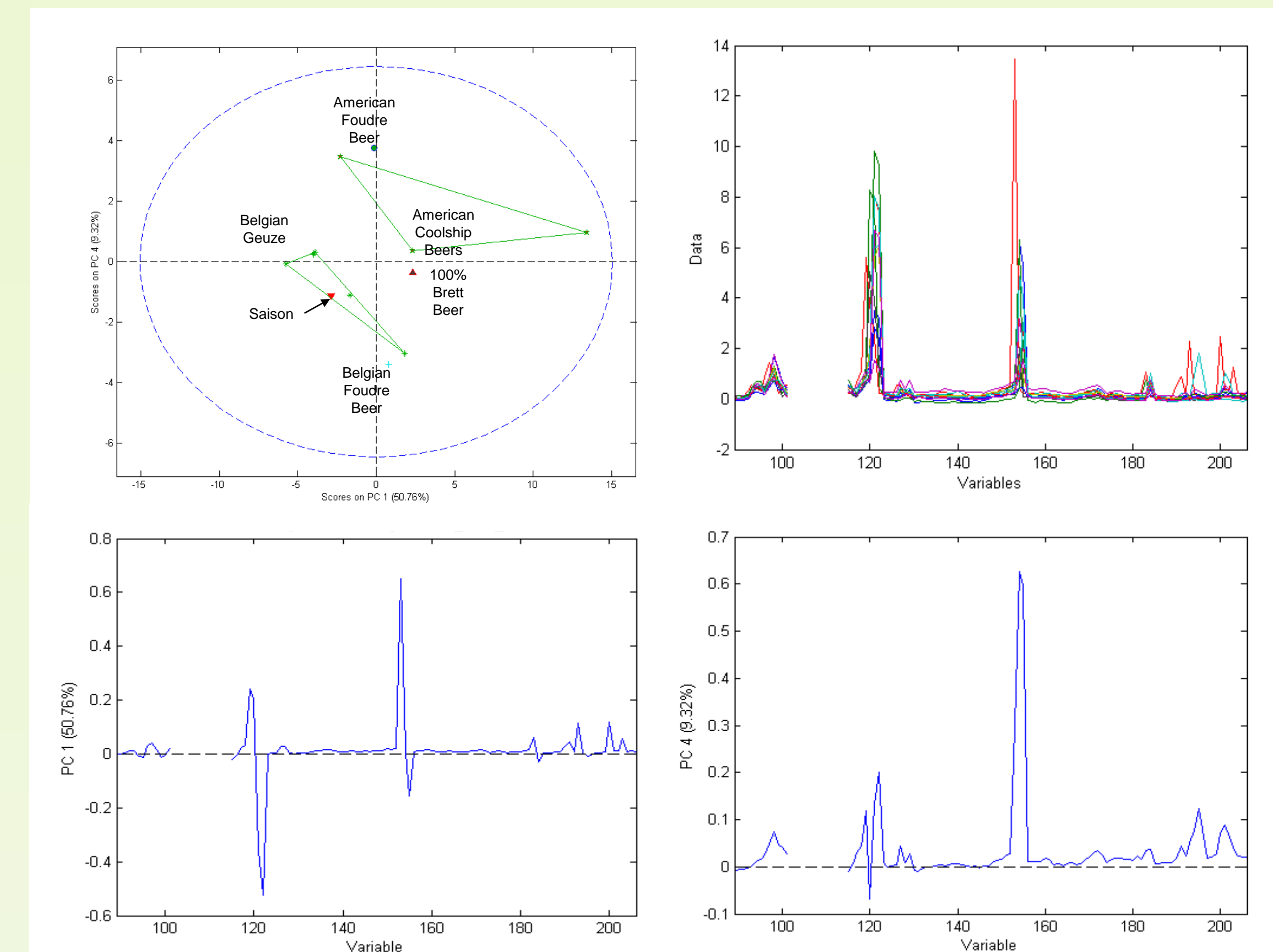


Figure 8: similar to segregation by whole freeze-dried spectra, American coolship beers were discriminated solely based on PCA of the acid region (top right, 0.8ppm-3.3ppm, excluding ethanol) (Top Left). Loadings on PC1 and PC4 indicate strong correlation based on acid content alone, specifically acetic acid (bottom row).

## Discussion

As displayed in Figures 4 & 5 NMR is an effective technique for the detection of many chemicals in beer, and the quantitation of the most important organic acids in spontaneously fermented beer. The most obvious difference based on the quantitation of organic acids is the relative amount of acetic acid between the American and Belgian lambic-styles. This may be indicative of a higher prevalence of *Acetobacter* species in the American terroire, or a unique metabolic profile of local *Brettanomyces spp.*, which can produce large acetic acid, which is largely dependent on the presence of oxygen<sup>5</sup>. Quantitation also reveals the presence of fruit acids, which are found in very high levels in beers containing added fruit.

The detection of species including amino acids, mono- and poly-saccharides, aromatics and organic acids contributes to the potential of the use of multivariate analysis in the discrimination of beers. As demonstrated in Figure 7 & 8, beers can be discriminated largely based on either sugars (Figure 7) or organic acids (Figure 8). The discrimination based on sugars was very effective, and highlighted the different sugar profiles of multiple beers over multiple styles. This may be the result of a combination of different ingredients as well as a difference between the metabolic output of a diverse and broad range fermentative organisms found in these beers.

## References

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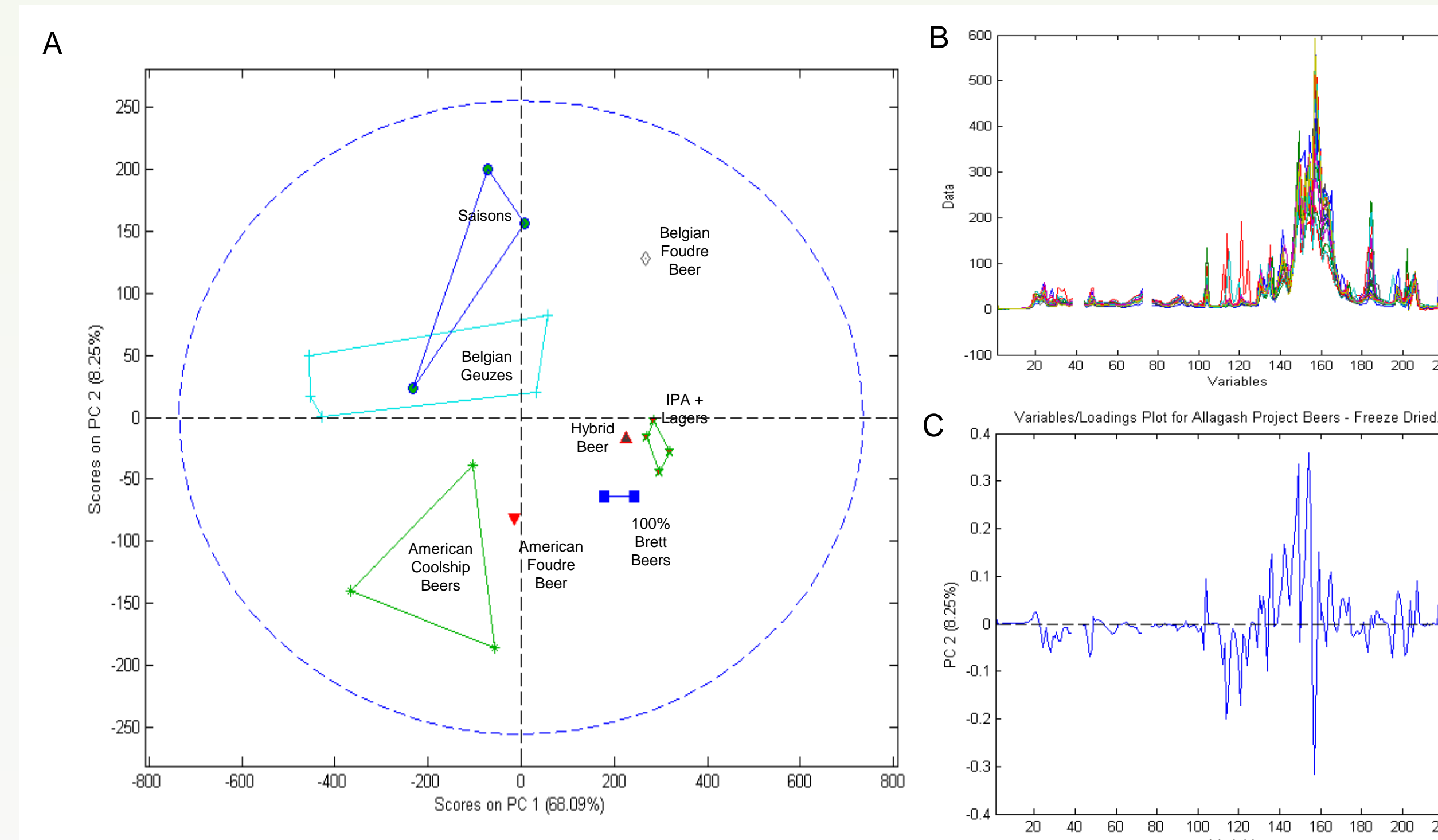


Figure 7: Results of Principal Component Analysis of 20 individual beers spanning 8 separate styles. Though only ~76% of the variance is explained in PCA-1 & PCA-2, samples segregate effectively by style (A). Integrals were taken from 0.5-9.5 ppm to every 0.02ppm, excluding ethanol, lactic acid, acetic acid and maleic acid (qNMR standard) and normalized to 10000. For this PCA the aromatic region was also excluded (~5.7ppm-9.5ppm) (B). Loadings on PCA 2 indicate discrimination based on sugars and non-volatile acids, highlighting potential metabolic differences between the variety of organisms involved in producing all of these beers (C).