From Mash to Bottle: Chemistry of the Brewing Process and NMR-based Quality Control

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Introduction

Beer is one of the oldest produced beverages in the world, and has been made from barley for at least 5,500 years[1]. Though modern beer is a far-cry from its ancient predecessor, the overall process remains the same: fermentation of sugars derived from saccharification of cereal grains.

The first step in modern brewing is the mash, in which crushed grains are mixed with hot water to activate native barley amylases. These enzymes then convert complex polysaccharides into smaller, fermentable oligosaccharides. The mixture of water and sugars (wort) is separated from the spent grains and transferred to a boiling kettle, and is then boiled. Hops are added to the boiling wort , which causes the isomerization of alpha acids, lending bitterness to beer, which balances the sweetness caused by residual sugars.

After boiling, the wort is cooled and transferred to a fermentation vessel, where a yeast culture is added to ferment the wort into "green beer." This unfinished beer is then filtered, carbonated, bottled/kegged and aged. This entire process is outlined below (Figure 1).

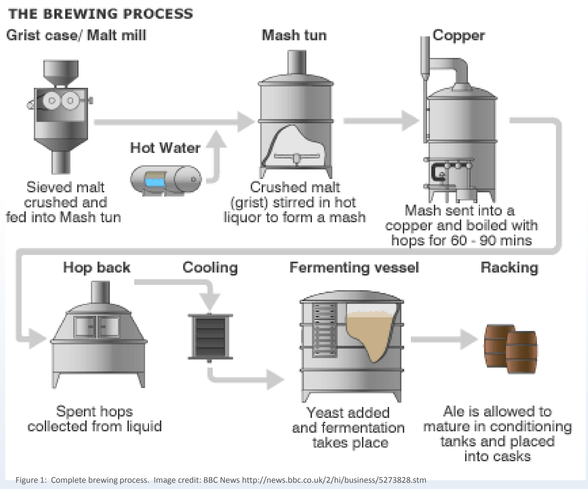

Figure 1: Complete brewing process. Image credit: BBC News http://news.bbc.co.uk/2/hi/business/5273828.stm

Because of the complexity of the complete brewing process, there are multiple opportunities for the application of NMR in brewery quality control. NMR and multivariate analysis have been applied as quality control measures previously[2,3], but never throughout the entire brewing process.

In conjunction with a commercial brewing company, this project seeks to utilize NMR and chemometrics to describe the full chemical changes that occur during the brewing process, as well as variations occurring between separate production lots.

Equipped with the knowledge of brewing process variables and their consequential chemical effects, brewers would be able to use NMR as a quality control measure to not only identify when production issues occur, but also where and why they occur.

The following data represent samples taken from multiple batches of the same beer. Labeled chemical species serve as representatives of identifiable compounds, along with illustrations of the inferences a chemist can draw by using these compounds.

Materials & Methods

Beer was acquired directly from an East coast brewery. Sample volumes were 175µL (straight runs) & 500µL (lyophilized) . Degassed beer samples were brought to a final sample volume of 0.75mL with deuterated water. Exactly 10mg of internal quantitation standard (maleic acid) was added to samples for quantitation.

Samples were run on a Mercury-VX 300 spectrometer operating at 299.681 MHz. Spectral Parameters: pw=67.5°, d1=7s, at=8s, T=27°C, nt=256 (straight runs) & nt=128 (lyophilized)

Spectra were processed in Mnova (ver. 8.2.0-12621) and Chemometrics were performed in Eigenvector (ver. 6.1)

Results & Discussion

Step 1: The Mash

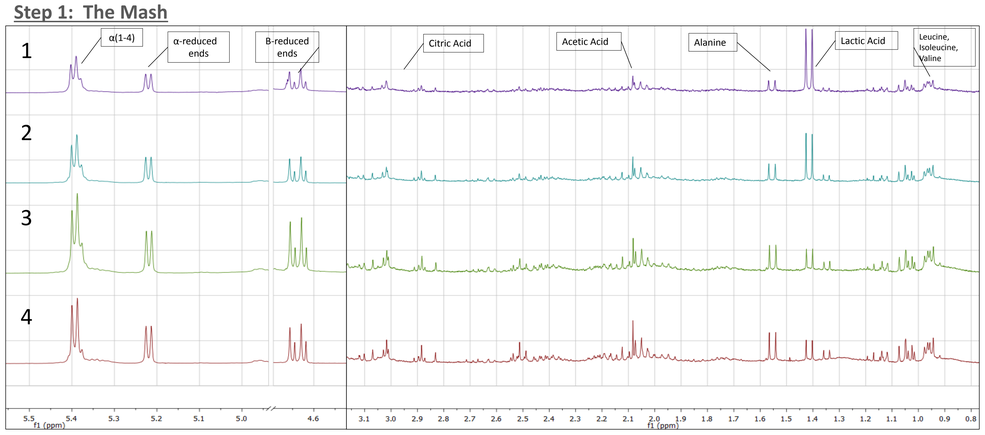

Figure 1: Changes in carbohydrates (left) and amino & organic acids (right) over the course of the mash. Mash-in (red/4), first runnings (green/3), sweet wort after 40 barrel run off (blue/2), end of sparge (purple/1). Relative to the initial mash(4), the first runnings (3) had larger amounts of all maltooligosaccharides (left panel), all of which decreased continuously to the end of the mash (1). The relative amounts of maltose (β-reduced ends, left doublet) and glucose (β-reduced ends, right doublet) were equivalent throughout steps 1-4. The amino and organic acids all decreased throughout steps 1-4, with the exception of lactic acid, which increased in relative concentration throughout the mash.

The increase in all carbohydrate resonances indicated that during the mash, amylase enzymes were responsible for the conversion of insoluble polysaccharides to water soluble oligosaccharides. Of these, malto-oligosaccharides experienced the largest relative increase, which could be used to determine mash conditions, as variations in temperature ranges influence proportions of mono- di- and trisaccharides present in the sweet wort.

Despite the relative constant concentration of the labeled organic and amino acids, lactic acid increased dramatically throughout the mash and sparge. The increasing amount of this acid could be indicative of an active population of lactic acid bacteria during the mash, which could potentially directly influence the flavor of a finished beer, as well as the pH of the wort, which could influence fermentation behavior of *S. cereviseae*.

Step 2: The Boil

No observable changes could be deciphered in samples pre- and post-boil.

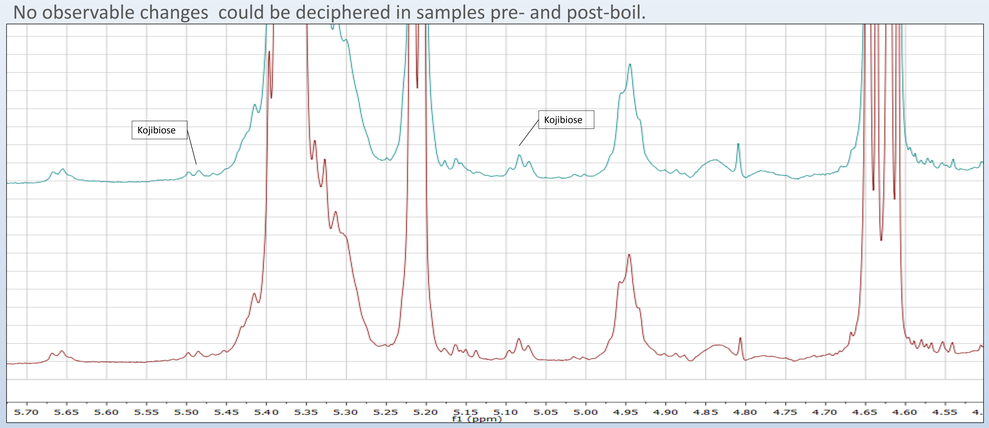

Figure 2: Spectra of carbohydrate region of samples pre- (top) and post- (bottom) boil. There were no significant differences in the relative amounts of any resonances displayed. Assigned resonances are identical to those in Figure 1, excepting the addition of kojibiose.

Though no direct trends were observed in pre- and post-boil samples, the potential exists that certain compounds could be used for quality control measures during the boil step. For example, kojibiose, a product of the carmelization of glucose, is a potential indicator for the condition of a beer while boiling, as excessive carmelization and browning can influence the color and flavor of a finished beer.

Step 3: Fermentation

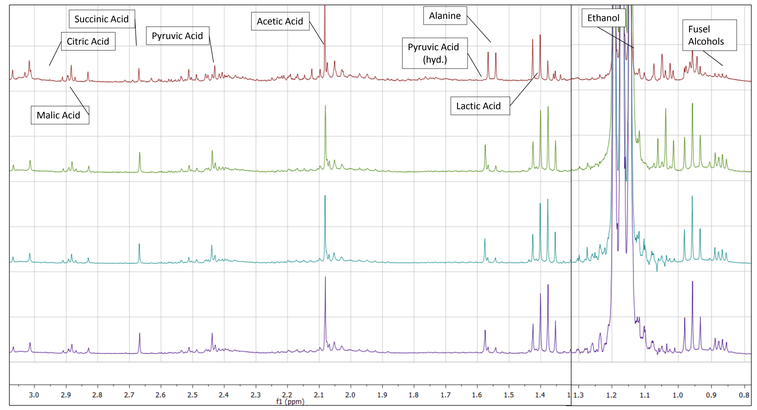

Figure 3: Changes occurring in beer during fermentation. Throughout fermentation, the relative amounts of pyruvic acid (and it's hydrate), acetic acid, lactic acid, ethanol, succinic acid and higher alcohols increased. Amino acids (alanine, leucine, isoleucine, valine) decreased throughout fermentation. Higher carboxylix acids, e.g. citric and malic acid remained the same throughout fermentation.

The fermentation of beer samples followed a predictable course as the concentrations of ethanol and fusel alcohols increased dramatically. Malt oligosaccharides decreased in a predictable way, as the glucose and maltose were preferentially fermented, leaving residual maltotriose and longer chain dextrins. This data gives insight into the final gravity of a beer, which impacts its body and mouthfeel.

In addition to the increase in alcohols, acetic and pyruvic acids increased in relative concentrations, both of which are intermediates in the ethanol fermentation pathway of *S. cerevesiae*. Because acetic acid is a precursor to the tricarboxylic acid cycle, and the relative concentrations of succinic, citric and malic acid stayed constant, it would be reasonable to infer that this process was completely anaerobic. The concentrations of these acids could be used to determine if there was an introduction of oxygen to the brewing stream.

The increase of lactic acid was marginal, and could be attributed to the metabolic activity of introduced yeast or resident lactic acid bacteria. In the event that production was due to lactic acid bacteria, the amounts were minimal and could be easily measured and assessed by NMR.

Chemometrics

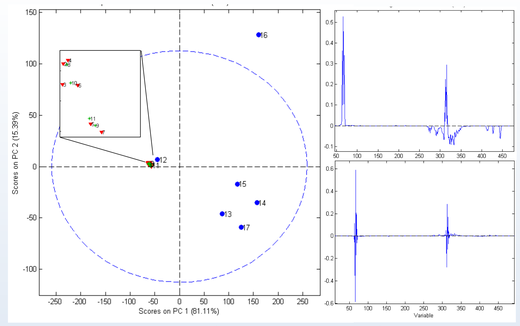

Figure 4: Principal Component Analysis of brewing stream. Samples were from two separate mash batches (green asterisks & red triangles [left]) which were then combined in the fermenter (blue circles [left]). Samples were as follows: 2 – Mash in A, 3 – Mash Out A, 4 – 1st Runnings A, 5 – Preboil A, 6 – Postboil A, 7 – Knockout A, 8 – 1st Runnings B, 9 – Preboil B, 10 – Postboil B, 11 – Knockout B, 12 – Fermenter A+B, 13 – 24hr Ferm, 14 – End Ferm, 15 – Prefilter, 16 – Carbonated, 17 – Bottled.

Principal component analysis of stream samples successfully resolved the three separate batches based largely on ethanol and oligosaccharide resonances. Within the expansion of Batch A and B, Mash In/Out and Preboiled samples were separated from Knockout and Postboiled Samples along PC2. The potential exists that this discrimination is related to protein and amino acid content of wort, as knockout and boiling of worts involves denaturation and precipitation of dissolved wort proteins. This could be correlated to free amino nitrogen content in wort, which has a direct influence on yeast fermentation behavior[4]. Despite data point discrimination, Batch A and Batch B occurred in close proximity in the PC1 x PC2 projection, suggesting that a simple PCA analysis may not be sensitive enough to discriminate initial brewing steps among multiple streams.

Future Work

The first step in future work is to obtain complete data sets for the entire brewing process. The acquisition of data completely documenting multiple streams of the brewing process would make chemometric-based quality control schemes possible.

The next step will be to build multivariate batch reaction models to identify outliers in the brewing process. Identification of samples and review of spectral data would make amelioration of process deficiencies possible, as many of the chemical species present in beer have specific origins and well defined impact on flavor. Instead of using simple PCA, modeling will focus on using multiway chemometrics, which are more useful in analyzing chemical changes batch and stream processes.

Finally, correlations and trends observed in spectral data will be considered in the context of brewing variables (e.g. wort gravity/color, mash temperatures, hop bittering levels [IBUs]) to improve the brewing process.

Acknowledgements

We would like to thank Franklin and the brewers responsible for furnishing these samples.

Citations

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2. Almeida, C. et. Al. (2006). Composition of Beer by ^{1}H NMR Spectroscopy: Effects of Brewing Site and Date of Production.
3. Duarte, I. F. (2004). Multivariate Analysis of NMR and FTIR Data as a Potential Tool for the Quality Control of Beer.
4. Lekkas, C. et. Al .(2007). Elucidation of the Role of Nitrogenous Wort Components in Yeast Fermentations.