077

WORLD BREWING CONGRESS 2016

The Chemical Fingerprint of Beer from a Single Experiment with Minimum Sample Preparation

A Rapid Quantitive Analysis by ^{1}H NMR Spectroscopy

John C. Edwards, Process NMR Associates, Danbury, CT 06810 USA

World Brewing Congress
August 13-17, 2016
Sheraton Downtown Denver
Denver, CO 80202, U.S.A.

Abstract: NMR Spectroscopy is the premier tool utilized by chemists to obtain detailed chemical information on molecular structure and is used extensively in molecular structure verification, chemical purity analysis, and complex mixture analysis. We have developed a quantitative NMR analysis that yields a chemical fingerprint that brewers can utilize to follow detailed variations in the chemistry observed in the various stages of the brewing process (malting, mashing, boiling, fermentation, ageing, and blending). The analysis observes all molecules in the beer at the same time and each molecular component (acids, alcohols, amino acids, malt-oligosaccharides) yields a unique spectral fingerprint pattern that is related to the structure of the molecule. Though the spectrum consists of a large number of overlapped individual fingerprints it is possible to identify and quantify individual components because many components have signals that appear at unique and specific points in the spectrum. The quantitative analysis is performed by comparing the area under the individual molecule signals to that of an internal standard (Maleic Acid 99%). Molecular components are quantified on a weight/volume basis in mg/L (parts-per million). Ethanol is also quantified on a %volume/volume basis.

The technique is not only applicable to the brewing process but is also being utilized to gain detailed chemical understanding of cider-making process, as well as the production of wine, mead, sake, spirits, and kombucha. Our laboratory has been developing this method with the help of a number of breweries following changes in batches of standard beers (Kolsch, Stout, Scots Ale, Barley Wine) as the brewing process is tweaked and changed over the course of 2 years. We have looked not just at finished beers but have studied dextrin solubility and chemistry of wort made from different malts, the effect of temperature on sour mashing, the effect of wild yeast and bacteria on various aspects of beer chemistry, as well as troubleshooting of “out of sensory target range” beers. The analysis requires very little sample preparation, has a large (orders of magnitude) linear concentration range of applicability and observes a large number of components in a single test that does not require constant re-calibration with expensive standards.

Sample Preparation: The only sample preparation step to be taken is a de-gassing procedure which we perform by repeated agitation in a vortex mixer.

Original beer sample analysis – 175µl of degassed was added to a 5mm NMR tube followed by 100µl of internal standard solution prepared such that the concentration was 10mg per 100ml. Finally, 475µl of D_2O (99.8%D) was added to the sample and the tube capped and agitated for 10 seconds.

Freeze dried beer samples – 1000µl of degassed beer was placed in a 2 dram vial and freeze dried in a Virtis Benchtop K. The entire dried sample was then dissolved in 650µl of D_2O and 100µl of maleic acid internal standard solution (10mg/100ml) added and the sample agitated before transfer to a 5mm NMR tube.

Standard Material – Internal Standard: Maleic Acid (99.0%) – Aldrich – Lot#SLBC1970V - 10mg/100µl solution in D_2O (99.9%D)

Experimental: ^{1}H NMR experiments were carried out on a Varian Mercury MVX-300 equipped with a 5mm Varian ATB probe operating at a resonance frequency of 299.67 MHz, Experiments were performed with a π/3 pulse with an 8 kHz spectral width collecting 64k over an 8 second acquisition time and with a 7 second relaxation delay. 64 transients were averaged to produce the final spectrum for analysis. In the final data processing the maleic acid resonance at 6.4 ppm was normalized to 10 so that a direct calculation could be made of all measured components on a mg/L basis. Analysis can be performed effectively on 300-800 MHz NMR systems.

Calculations: The following volatile components were always obtained on a 175 µl beer sample as the freeze drying process would compromise the amount present: ethanol, acetic acid, fusel alcohols (isobutanol, isopentanol (isoamyl alcohol), and 1-propanol) ethyl acetate, methanol. The calculation utilized to quantify these components was:

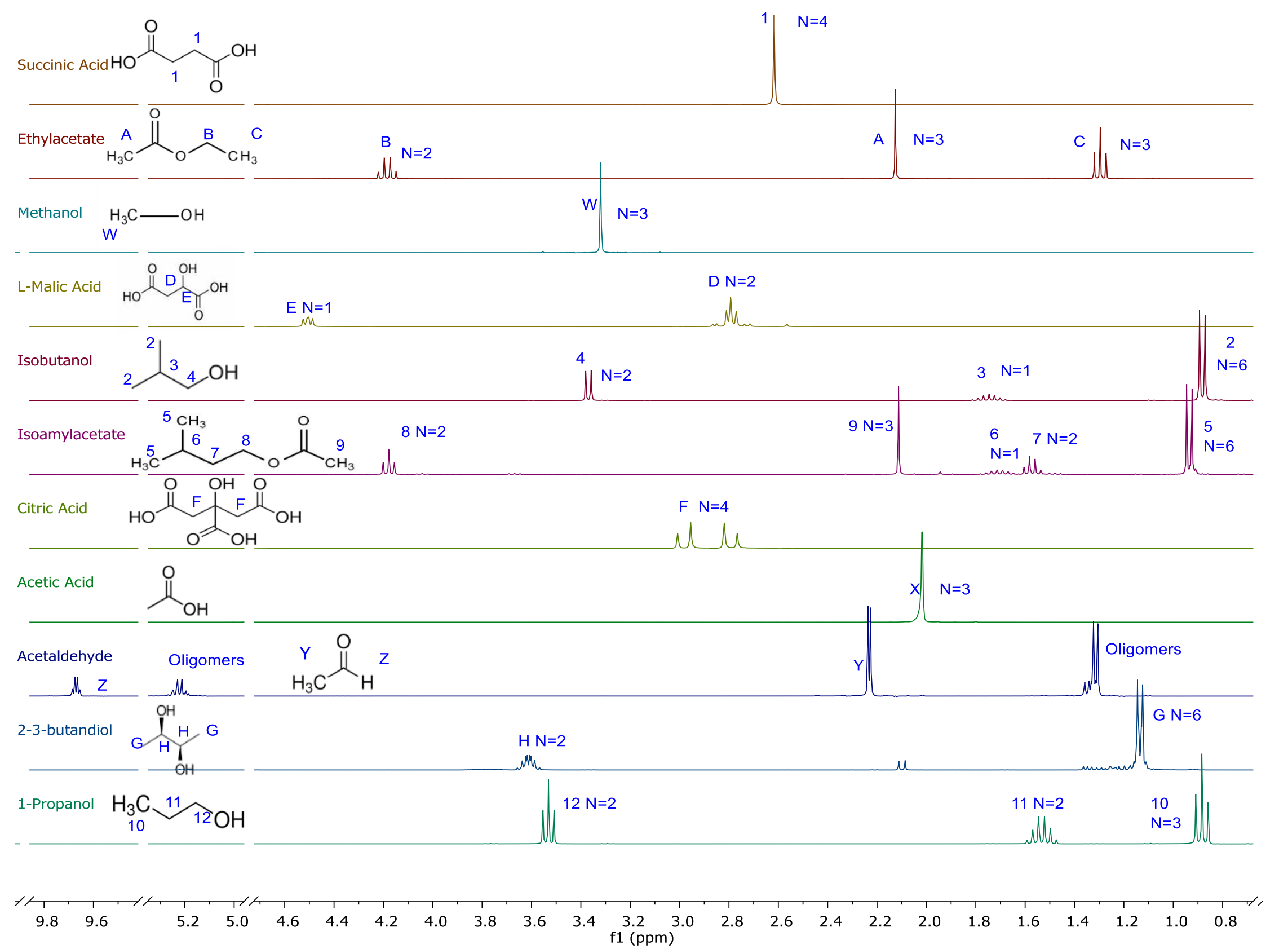
$$\text{Component mg/L} = 0.99 * 10\text{ mg} * ((I_{comp} / N_{comp}) / 50) * (Mw_{comp} / 116.1) * (1{,}000{,}000 / 175) \qquad \text{Eq. 1}$$

Where, wt of maleic acid (MA) = 10mg, I_{comp} = Integration of component resonance, N_{comp} = number of protons integrated, $I_{MA}/N_{MA} = 50$ (MA integral set as 100), Mw_{comp} = molecular weight of component molecule, M_W of MA = 116.1 amu, the 1,000,000/175 factor rectifies the volumetric component of the calculation to allow mg/L to be calculated. 0.99 represents the 99% purity of the maleic acid standard.

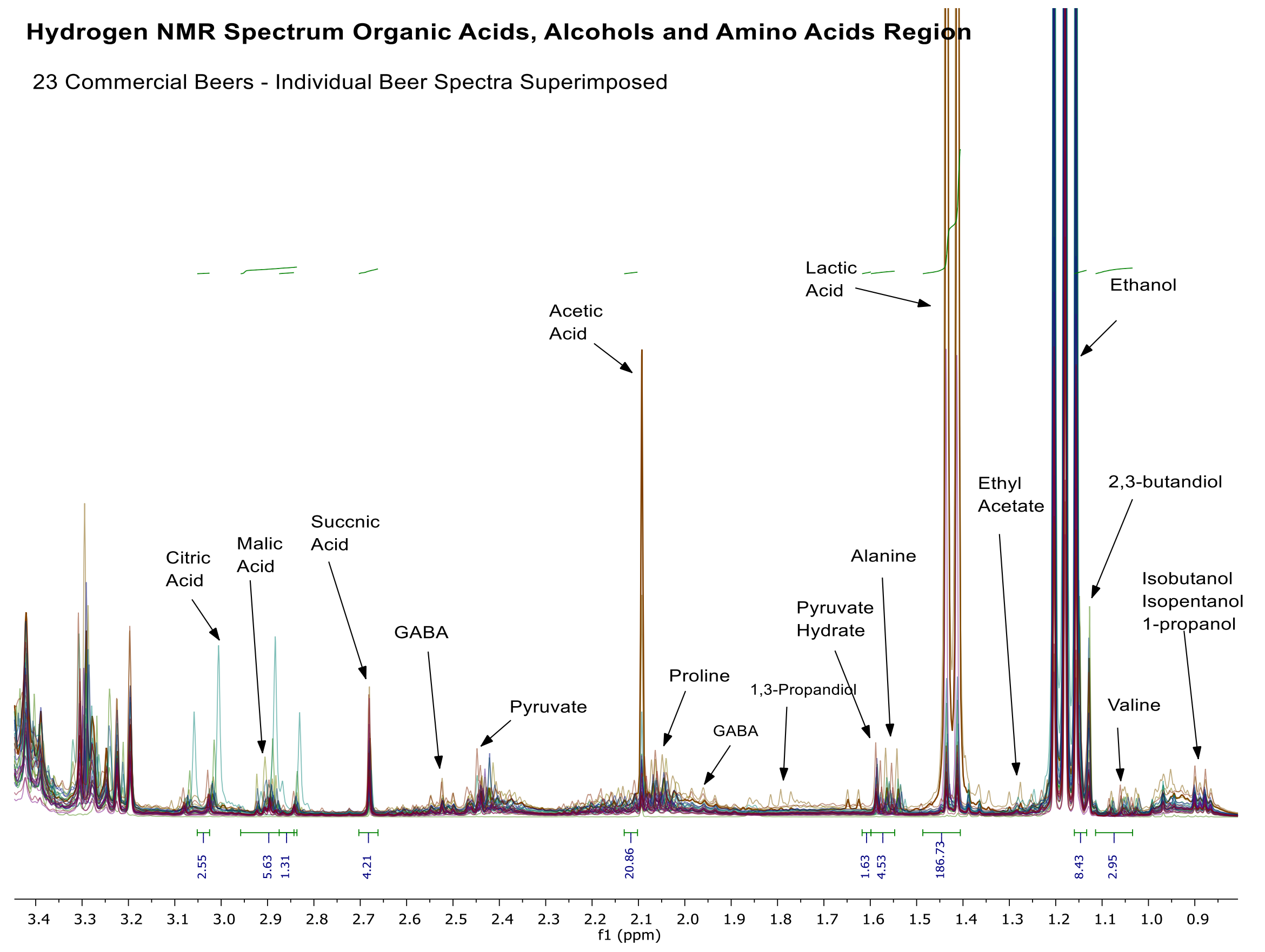
The following non-volatile components were always obtained on a 1000 µl freeze dried sample: lactic acid, succinic acid, malic acid, citrate, various amino acids, glucose, malt and carbohydrates, glycerol. The calculation utilized to quantify these components was:

$$\text{Component mg/L} = 0.99 * 10\text{ mg} * ((I_{comp} / N_{comp}) / 50) * (Mw_{comp}/ 116.1) * (1{,}000{,}000 / 1{,}000) \qquad \text{Eq. 2}$$

Where, wt of MA = 10 mg, I_{comp} = Integration of component resonance, N_{comp} = number of protons integrated, $I_{MA}/N_{MA} = 50$ (MA integral set at 100), Mw_{comp} = molecular weight of component molecule, M_W of MA = 116.1 amu, the 1,000,000/1,000 factor rectifies the volumetric component of the calculation to allow mg/L to be calculated. 0.99 represents the 99% purity of the maleic acid standard.



^{1}H NMR spectra of a number individual molecular components found in fermented beverages. Molecular assignments of the various NMR peaks representing proton chemistries present in each molecule are show along with the molar number of protons that each peak represents. These pure component spectra of all individual molecules found in beer are superimposed on each other in the final NMR analysis. However, a knowledge of the component spectra allows the observation of unique NMR signals for many molecular components allowing the identification and quantification of the components present in the complex mixture that is the beer.



Organic Acid Analysis – Useful particularly in Beer spoilage and Sour Beer Brewing and Ageing:

The figure above is a superposition of 23 spectra showing the part of the proton spectrum where signals from alcohols, acids, and amino acids are observed. The assignments of the various chemical components are labelled. Different hydrogen chemistry is observed on the X-axis showing $CH/CH_2/CH_3$ signals that appear as single, double, triple and quadruple peaks depending on the molecular structure of the molecule. Note that the NMR spectrum axis shows units of ppm – this is not a weight value but rather a normalized frequency scale which allows spectra from different NMR instruments to be directly compared. The data shown in the plot to the right shows the organic acid distribution obtained on 23 commercial beer samples.

We have utilized the Organic Acid profiles to determine if unexpected lactobacillus activity has occurred during the mashing, fermentation or ageing of various beer styles. It can be utilized to obtain organic acid profiles for 1/2/3 year old geuze blending. The glycerol conversion metabolism of wild yeast and bacteria strains can also be readily observed by the observation of 1,3-propandiol after high conversion of malt to alcohol. The detailed chemistry is also of interest in the development of wild fermentation understanding as it provides a detailed chemistry window of the action of various yeast and bacteria strains on standard worts.

Beer Components Quantified

- Alcohols: Ethanol, Propanol, iso-Butanol, iso-Pentanol, Methanol
- Organic Acids: Lactic, Acetic, Malic, Succinic, Pyruvic, Pyruvic-Hydrate, Tartaric, Formic
- Carbohydrates: Maltose, Glucose, Malto-oligosaccharides with Degree of Polymerization (Dp), Fructose, Isomaltose, Lactose, Sucrose, Kojibiose, Arabinoxylan, Limit Dextrin
- Amino Acids: Alanine, Valine, Proline, GABA, Tyrosine, Tryptophan, Histidine, Glutamate, Asparagine, Phenylalanine
- Nucleotides: Uridine, ATP
- Other Metabolites: Glycerol, Acetoin, 2,3-Butandiol, Diacetyl, Ethyl-Acetate, 1,3-Propandiol
- Additives: Theobromine (Cocoa), Caffeine (Coffee or Tea), Sorbic Acid (Preservative), Tartaric Acid (Grape Must)
- Experiment Limitations: Components present at less than 5-10 mg/L (ppm) are difficult to observe and quantify. Example: Hop components and low concentration defect components are not directly observed unless present at high concentrations.

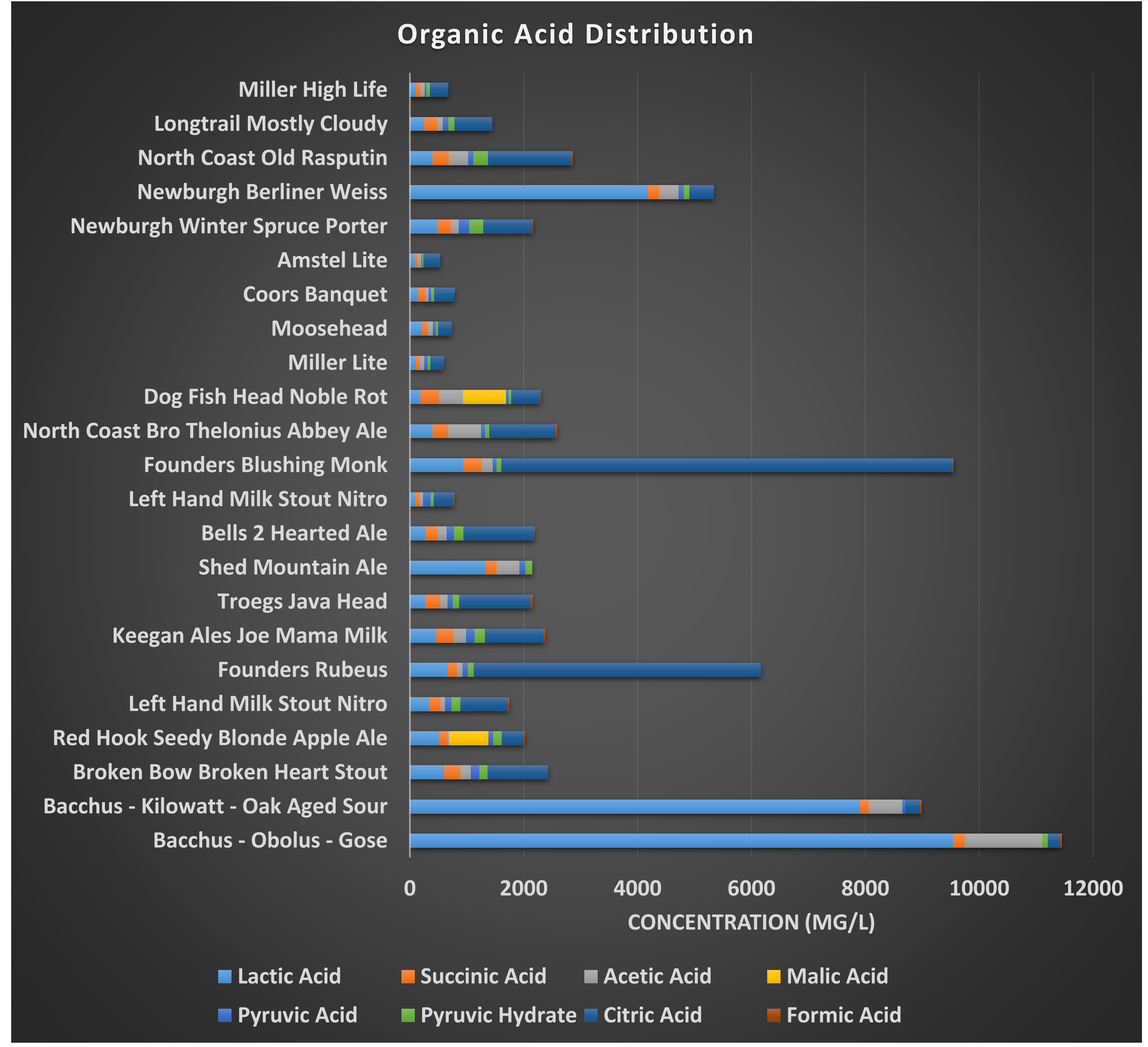
Alcohol and Diol Analysis: The NMR analysis is routinely utilized to obtain the ethanol content of degassed beer samples. The analysis can be utilized to quantify ethanol across the entire range expected values from 0.05 %ABV in non-alcoholic beers up to 50%ABV produced by specialized brewing yeasts. The response is entirely linear and results correlate closely with other techniques such as head space GC, HPLC, ASBC distillations, and density meter-based approaches. The figure to the right shows a comparison of the NMR calculated ethanol content compared to the bottle or tapdeclared values for the 23 beers presented here. The technique can also quantify methanol, fusel alcohols (1-propanol, isobutanol, isopentanol) as well as diols such as 1,3-propandiol, 2,3-butandiol, and finally glycerol produced during the fermentation process.

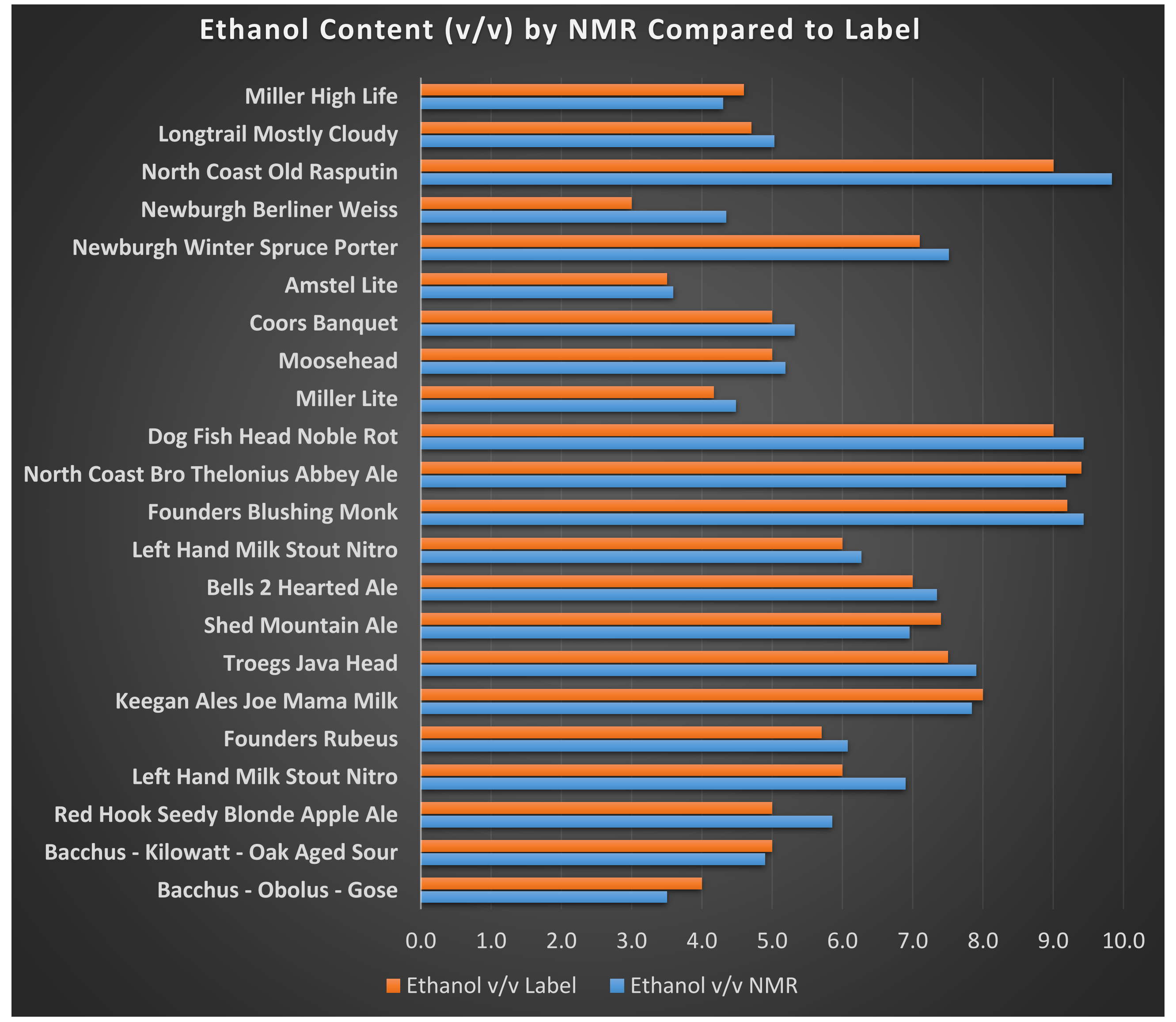
Amino Acids and Nucleotides: The NMR technique can be used to quantify a large number of amino acids and nucleotides providing information on yeast accessible nitrogen and information on the protein content of the beers. A graphic is provided showing the quantification of a large number of amino acids in the 23 commercial beers presented here.

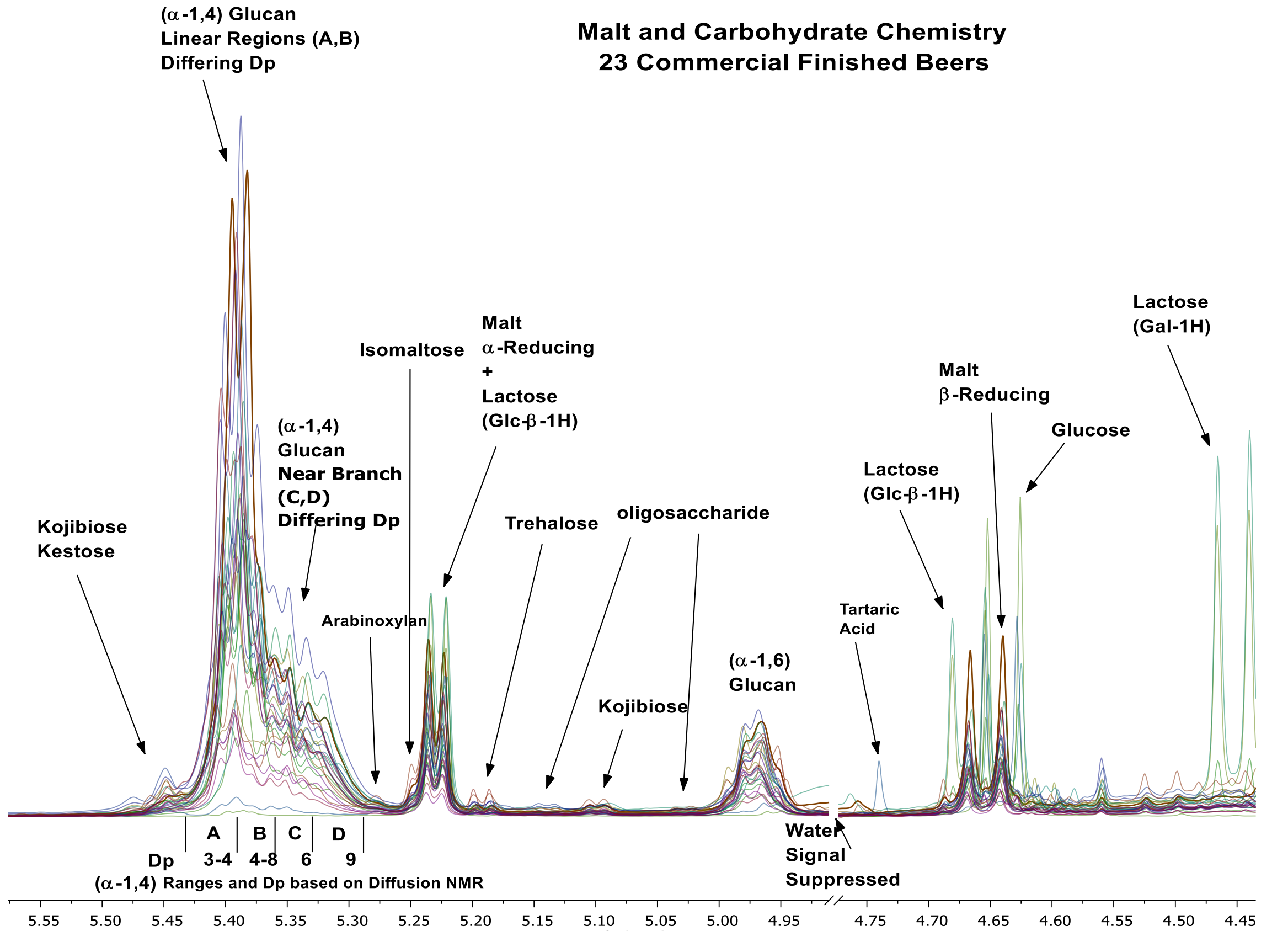
Nutritional Information: As the detailed chemical fingerprint has been produced it is a straightforward calculation to provide calorie values for the beers that are analyzed. The contribution of the individual beer components (Ethanol/Carbs/Amino Acids/Organic Acids) to the total calorie count can also be provided. Another aspect of NMR analysis that we are currently investigating is the simultaneous observation of sodium in beer samples to obtain a quantitative value for sodium for labelling. (^{23}Na is a highly NMR active nucleus that can be quantified down to ppm levels in a relatively short time with minimal sample preparation).

Adjunct Chemistry: The ^{1}H NMR analysis can also be utilized to observe the quantity of adjunct chemistry present in a beer sample. Beer that contains wine must will show observable quantities of malic acid and tartaric acid. Apple ales will also show quantifiable malic acid content. Cocoa nibs provides a source of Theobromine that can be analyzed by the NMR, and coffee addition provides caffeine that can be observed and quantified. Addition of rice and other sources of carbohydrate can be observed in changes of dextrin character.

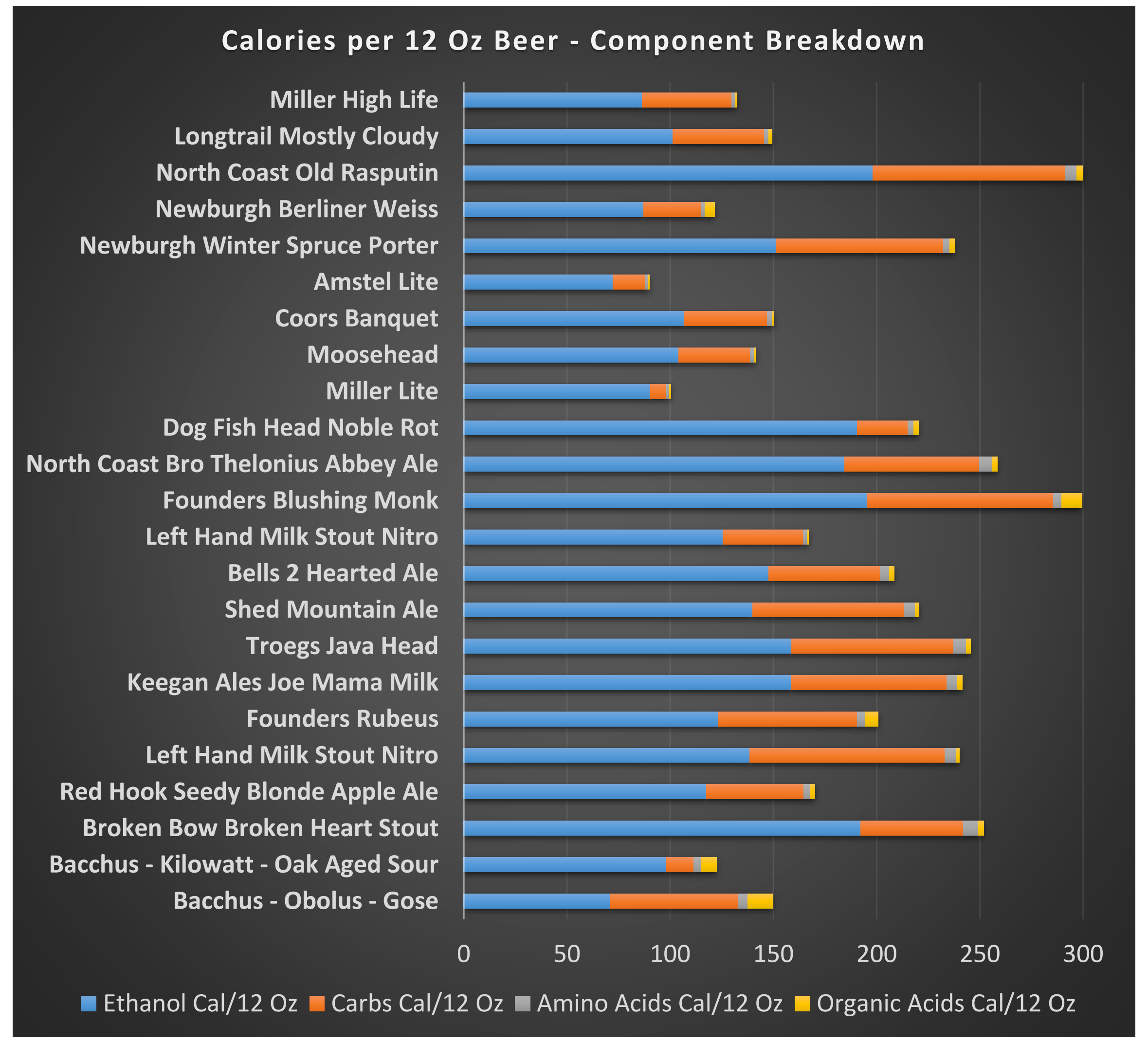
Hops and Lipids: ^{1}H NMR approaches have been developed to investigate the hop and lipid component chemistries. However these approaches are complicated by the very low concentrations and variable solubilities of these two components. These approaches will be the subject of further research.

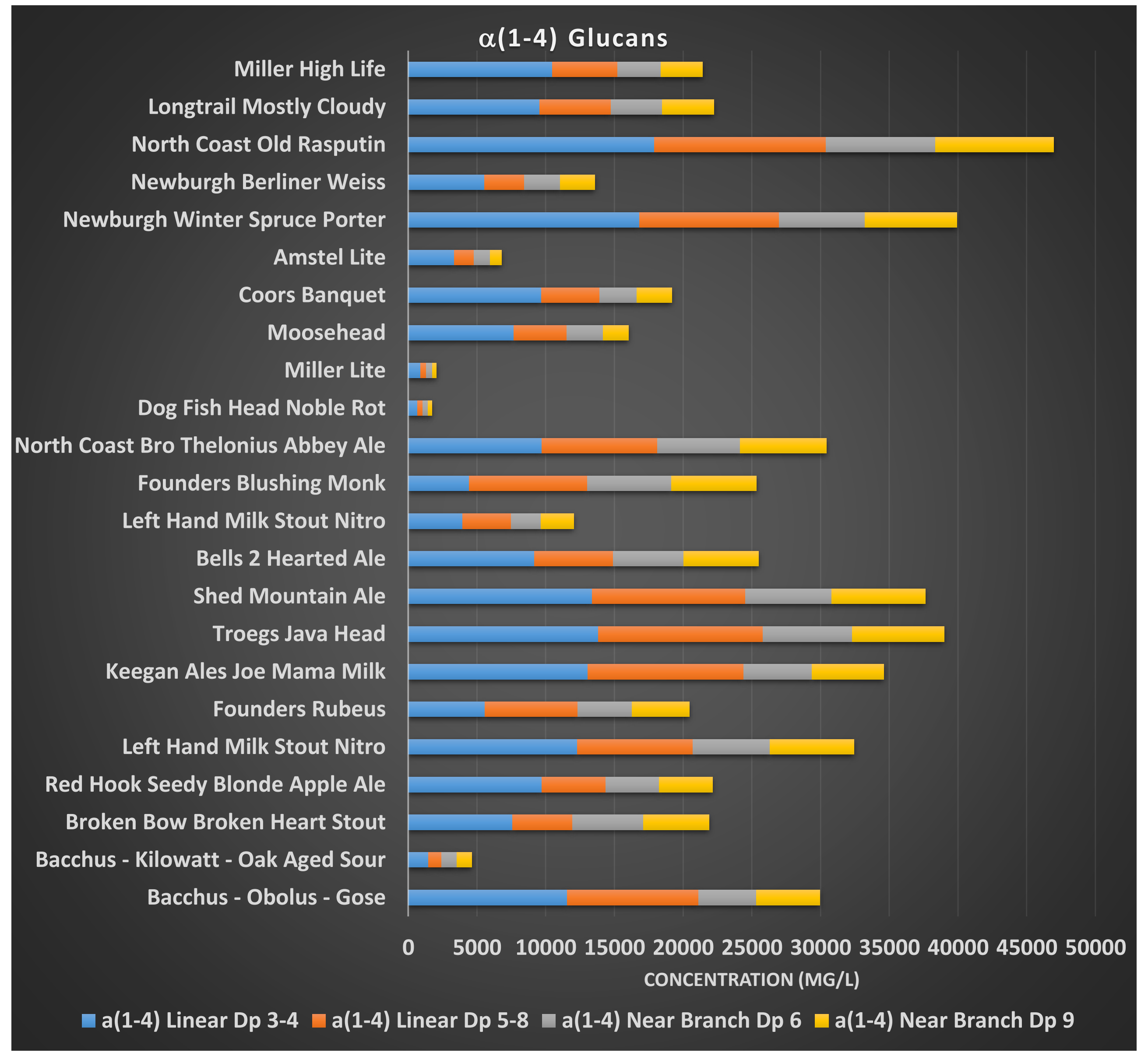


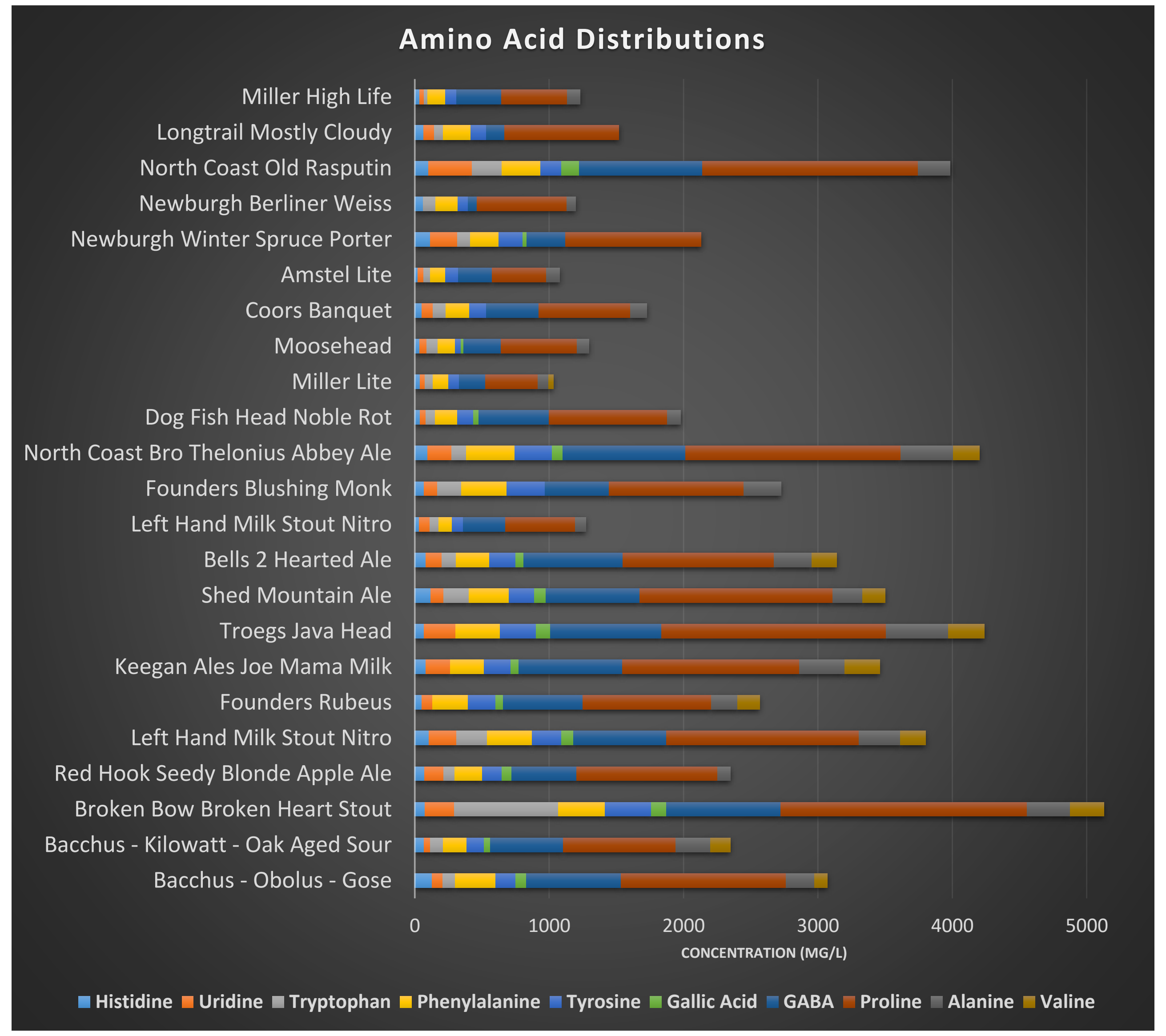




Malt, Dextrin, and Carbohydrate Chemistry: The Figure above shows the expansion of the anomeric hydrogen region of the NMR spectrum covering a range malt contents provided by 23 commercial beers. This area of the spectrum provides the quantitative fingerprint of the carbohydrate and malt-oligosaccharide chemistry of the beer. The 4.9 to 5.5 ppm region of the spectrum contains signals from a-glucan malto-oligosaccharides and limit dextrin containing α(1-4) and α(1-6) glycosidic linkages. The 4.4 to 4.75 ppm region contains signals from β-glucan carbohydrate components. In finished beers the concentration of these components gives an indication of the incomplete conversion of starch to yeast fermentable carbohydrates. Differences in the distribution of starch-derived oligosaccharides reflect the use of different malting conditions, mashing protocols, yeast strains, and enzymatic processes that effect the degree of starch breakdown. In the analysis of mash and wort analysis the ratio of fermentable sugar to non-fermentable sugar can be calculated. The degree of polymerization of malto-oligosaccharides can be determined and the relative distribution of oligosaccharide components can be obtained. The effect of mashing and boiling conditions can be monitored and the concentration of various malt components quantified at different time points in the process allowing optimization of time and energy use. As an example of the variability that is seen in different commercial beers the figure to the right shows the α(1-4) glucan component comparison for 23 beers with a breakdown into linear and branched dextrins of differing degrees of polymerization (Dp) ranging from 3 to 9 monomer units.







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