The Craze for the Haze: How Dry Hopping Influences the Metabolomics of Beer

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ABSTRACT

Purpose: This collaboration with Omega Yeast is designed to show how the use of high-resolution mass spectrometry can enlighten small molecule changes within a complex matrix. Scientists are always seeking to know more about what is present in their samples and how their experimental conditions are affecting these compounds. Beer's flavor is generated from a combination of yeast's metabolic activity, various plant derived compound, and the initial source for starch. Using information that was discovered in previous experiments, hop timing was targeted to show a relationship between the dry hop time and the hazy quality of beer.

Methods: The untargeted methods used here leveraged the newest technology in the mass spectrometry arena. The Thermo Scientific™ Orbitrap Exploris™ 240 mass spectrometer was used to analyze beer made from a single yeast strain, barley, and varying dry hopping addition times. This untargeted metabolomics approach allowed for the unbiased detection of small molecules within the samples. Since samples were hazy from suspended proteins, it was removed by an excess of chilled methanol prior to analysis. Internal standards were used as quality controls, and pooled samples were used to normalize any batch effects across the run as well as creating ddMS2 data. Data was processed using Thermo Scientific™ Compound Discoverer™ 3.3 software, which facilitated library searching, retention time matching, compound annotation, multivariate analysis, and pathway mapping withing a single piece of software.

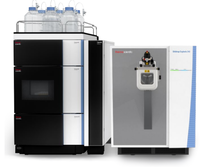
Results: The metabolite profiles based on varying hop addition times are shown to generate unique profiles through multivariate analysis. After applying a strict set of filtering criteria, the data set was reduced to <5000 different compounds that had low variation across the QC samples, less than 30% CV for each group, and all compounds had an associated MS2 spectra. Within this set, it was possible to identify up to 10 major bitter alpha acids that were extracted from the hops during processing. This was done by matching retention time and fragmentation pattern to a set of standards. Specific changes can be seen in lupulones, humulones, and rho-acids. The changes in the specific bitter acids are responsible for the novel flavors produced in dry hopped beers. Significant changes are also seen in amino acid levels especially the phenylalanine, tryptophan, and tyrosine.

INTRODUCTION

Over the past few years, there has been an explosion in small craft breweries popping up, creating novel brews, and redefining what average people drink. Everyone is clamoring for beers with unique flavor attributes especially hazy beers. This has also driven many individuals to pick up home brewing in search of that new esoteric hazy beer. In turn, this demand had driven the suppliers of yeast strains to step up and start marketing new yeast strains. Omega Yeast is at the forefront of this movement and has been targeting yeast strains that produce beers extra hazy qualities. They are also scientifically curious about what happens to the metabolome of these new hazier beers. This project is a collaboration between Laura Burns and Lance Shaner at Omega Yeast, and Christopher Bolcato at Thermo Fisher Scientific (Figure 1). To fully understand these hazy beers, Chis is analyzing the same samples for protein profiling.

Figure 1- Collaborators from Omega Yeast and Thermo Fisher Scientific

Figure 2- Vanquish UHPLC and Orbitrap Exploris 240 used for analysis



MATERIALS AND METHODS

Sample Preparation: 2 different dry hopping time were used: 24 and 48 hours. There were 5 biological replicates, and 3 technical replicates created for all both time points. Samples were collected upon completion and immediately frozen at -80 °C until extraction. Protein precipitation was done with a 3x volume of chilled methanol. A sample preparation workflow is shown in figure 5.

Test Method(s): All samples were separated using a Vanquish Flex UHPLC with a Hypersil GOLD™ (100 x2.1 mm 2.6 µm) column (Figure 6) and analyzed using an Orbitrap Exploris 240 mass spectrometer. Data was collected in high resolution full scan (120k) and data depended MS2 using Acquire X. (Figure 7).

Data Analysis: Compound Discoverer 3.3 software was used to process all data files. (Figure 8)

SAMPLE PREPARATION

Figure 3 –Example of how dry hopping changes the haze character of beer.

Figure 4- Set of humulone standards use for compound confirmation.

Figure 5- Sample preparation workflow

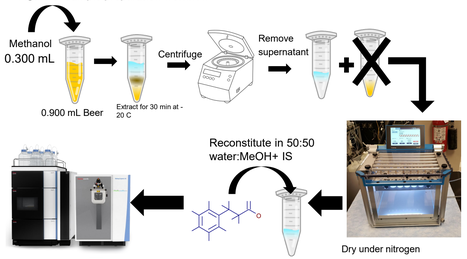
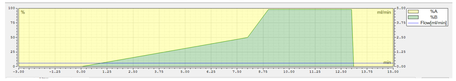


Figure 6- Liquid chromatographic separation profile



Hypersil GOLD column (100 x 2.1 mm, 1.9 µm)
Mobile Phases: 0.1% FA in (A) H2O / (B) ACN
Flow rate: 0.300 mL / min
2 µL injection

No	Time	Flow [mL/min]	%B	Curve
1	-3.000	Equilibration		
2	-2.000	0.300	0.0	5
3	*New Row*			
4	0.000	Run		
5	0.000	0.300	0.0	5
6	8.000	0.300	50.0	5
7	9.000	0.300	98.0	5
8	13.000	0.300	98.0	5
9	13.100	0.300	0.0	5
10	*New Row*			
11	15.000	Stop Run		

LC-MS METHODS

Figure 7- Mass Spectrometer settings for MS1 and FS-ddMS2 analyses

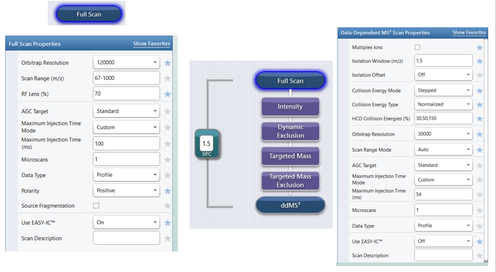
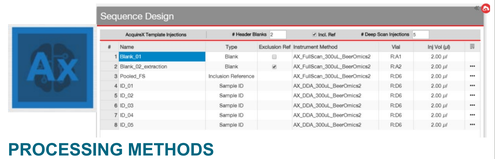
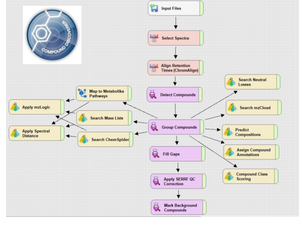


Figure 7- Deep Scan Acquire X sequence



PROCESSING METHODS

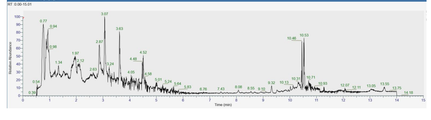
Figure 8- Compound Discoverer 3.3 analysis workflow



Compound Discoverer 3.3 Highlighted nodes

- Align RT (ChromAlign)
- Detect Compounds (new)
- Apply SERF QC Correction (batch effects)
- Mass list (17 Humulones with RT, and internal standard)
- Compound Class Scoring (catechins)

Figure 9- Example for TIC for BeerOmics sample



Results

Using the new detection algorithm that is present in Compound Discoverer 3.3, it is now possible to detect compounds with a very low intensity threshold, then automatically remove peaks with poor peak quality. This translates into the detection of more peaks and less worry about missing low abundant peaks as well as this being done 5x faster. Peak quality filters allow for the removal of compounds persistent across the chromatography, have long tails, or are very jagged peaks.

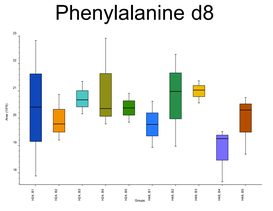
This data set had initially detected 12,186 compounds and after applying a peak quality threshold of 4.5, and a pooled QC based normalization filter, 4,141 were remaining and used for all subsequent analyses.

Data quality was assessed based on the reproducibility of the labeled internal standard, phenylalanine d8. This compound was spiked into all samples at 500 nM. Peak areas, averages, and CV% was calculated based on 3 replicates per biological group. Coefficient of variation for 9 of the 10 groups were <10% (Table 1, and Figure 10).

Table 1. Phenylalanine variation per replicate group

Treatment time	Biological Group	CV %
Hour 24	1	12%
	2	4%
	3	3%
	4	8%
	5	4%
Hour 48	1	7%
	2	8%
	3	2%
	4	5%
	5	5%

Figure 10- Box and whisker plot of Phe d8 for each biological replicate group analyzed.



Five Acquire X deep scan MS2 injections were used to collect fragmentation data from the pooled QC sample. All MS2 scans were automatically assigned to the appropriate compounds. From the remaining 4,141 compounds 86% of those compounds had an MS2 assigned for the M+H+ ion, while 6% of compounds didn't have MS2 data (Figure 11). This greatly enhances the ability to either annotate compounds based on library matches or us FiSH scoring.

Figure 11- Pie chart showing percentage of compounds with MS2 spectra.

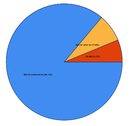
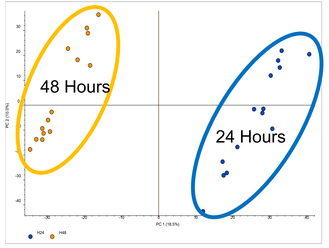


Figure 12- Principal Component Analysis (PCA) showing group separation between 24 and 48 hour hopped beers



Results (continued)

PCA (Figure 12) creates an unsupervised metric to show there is a distinct metabolite profile between the 24 and 48 hour hopped beers. PC1 explains 18.5% of the variation while PC2 only explains 10%. There also appears to be some intragroup variation as represented by the wide distribution of the orange and blue circles.

A volcano plot was used to initially screen the untargeted data for compounds that show large (>|4x|) and significant fold changes (p value<0.5). 38 unique compounds were noted as being decreased in the 24/48 comparison; thus these compounds were in higher abundances in the 48 dry hopped samples.

Figure 13- Volcano plot- a negative fold change (green), a positive fold change (red)

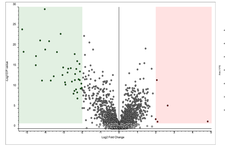


Figure 14-Comparative analysis for 2 compounds that show a significant increase

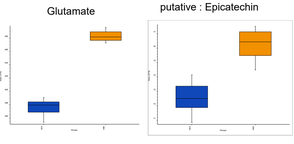
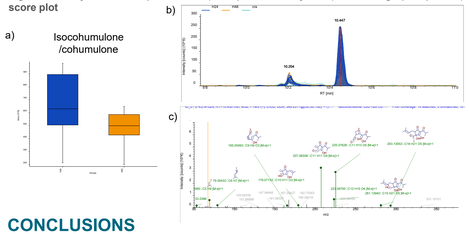


Figure 15-Analysis for iso alpha acids. a) comparative analysis, b) chromatographic peak, c) FiSH score plot



CONCLUSIONS

This hop time study of beer has shown it is possible to use semi targeted metabolomics to being understanding how the timing of dry hopping in beers effect the overall small molecule composition of the final product. Just by comparing two different times, 24 hours and 48 hours, distinct changes in specific compound can be seen including compounds glutamate and various catechins. It's interesting to point out that regardless of dry hop timing, the quantity of several humulones didn't change significantly. Future work includes expanding this experiment to include multiple other time points to capture the fluctuation of these specific bitter acids and their related compounds. This will help brewers better understand when to dry hop beers to create the intended flavors and taste.

ACKNOWLEDGEMENTS

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TRADEMARKS/LICENSING

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