Plants, Food, Environment and Microbes

Exploring the Effects of Yeast Strain and Hop Addition Time on the Metabolomics of Beer

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Abstract

Purpose: Brewers all around the world are interested in uncovering the secret to a perfect India Pale Ale (IPA). In this collaboration with Omega Yeast, we explore how unique yeast strains and hop addition time can influence the metabolomics of beer. Understanding how compounds change with these parameters will help brewers perfect their recipes to create distinct flavors and aromas.

Methods: Beer samples were analyzed with a Thermo Scientific™ Vanquish™ Flex UHPLC system and a Thermo Scientific™ IQ-X™ Tribrid mass spectrometer. All the samples were run in high-resolution full scan and then pooled to collect MS2 spectra using the Acquire X advanced deep scan workflow. Data were processed using Thermo Scientific™ Compound Discoverer™ 3.3 software for unknown identification and Thermo Scientific™ TraceFinder™ 5.1 for quantitative analysis.

Results: A single injection simultaneous quantitation and discovery (SQUAD) metabolomics workflow was developed for accurate quantitation of target compounds and confident detection of unknown metabolites in a single injection. The data analysis showed the grouping of specific yeast strains and increasing trends for various compounds with hop addition time.

Introduction

Beer is a complex beverage made by combining yeast, malt, hops, and water (Figure 1). There are several parameters that can be changed during the brewing process and drastically impact the type and quality of the beer. The yeast strain is one of these parameters and brewers around the world rely on yeast manufacturers like Omega Yeast to produce metabolically strong yeast that will give them consistent fermentation. In this study, we collaborated with the scientists at Omega Yeast to explore the effects of yeast strain and hop addition time on the metabolomics of beer using orbitrap-based LC-MS.

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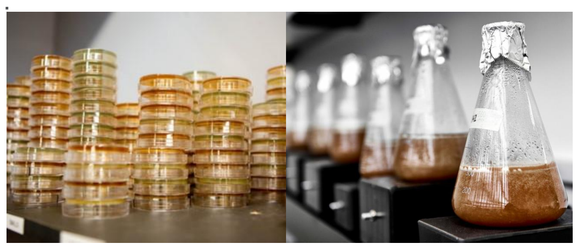


Figure 1. Fermentation During the Brewing Process

Materials and methods

Sample Preparation: A total of 46 beer samples were collected and stored at -80ºC until analysis by LC-MS. The samples were divided into two groups to examine the effects of yeast strain and dry hop addition time. The first group included 4 yeast strains with 3 time points and the second group included 1 yeast strain with 7 time points. Figure 2 shows the extraction protocol for each sample.

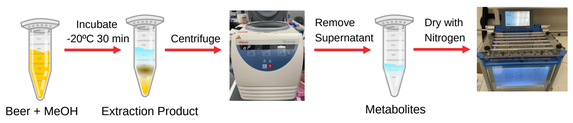


Figure 2. Sample extraction protocol

LC-MS Methods: All samples were reconstituted in 300 µl of 50/50 MeOH/H2O containing 500 nM of a [13]C[15]N isotope-labeled amino acid mix and then subjected to LC-MS analysis. Metabolites were separated on a Hypersil Gold™ (100 x 2.1 mm, 1.9 µm) column using a Vanquish Flex™ UHPLC system and analyzed using an Orbitrap IQ-X™ Tribrid™ mass spectrometer. All samples were run in full scan using high resolution (i.e., 120k) and then pooled to collect data-dependent MS^n spectra using AcquireX (Figure 3).

Data Analysis: A simultaneous quantitation and discovery (SQUAD) metabolomics workflow was developed using Compound Discoverer™ 3.3 and TraceFinder™ 5.1 software to perform targeted quantitation of amino acids, detect polyphenolic compounds and identify unknowns (Figure 4).

Vanquish Flex™ UHPLC

Hypersil Gold™ Column
(2.1 x 150 mm, 1.9 µm)
Flow rate: 0.300 mL/min
Injection Volume: 2 µL
Mobile Phases
A: H20 + 0.1% FA
B: MeOH + 0.1 % FA

Time (min)	%B
0	0
8	50
9	98
13	98
13.1	0
18	0

Orbitrap IQ-X™ Tribrid™ Mass Spectrometer

MS1

Resolution=120,000
Scan range= 67-1000 m/z

FS-ddMS2

Resolution=60,000/30,000
Cycle time= 1.2 S

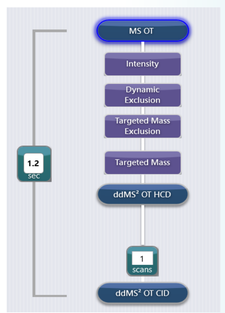
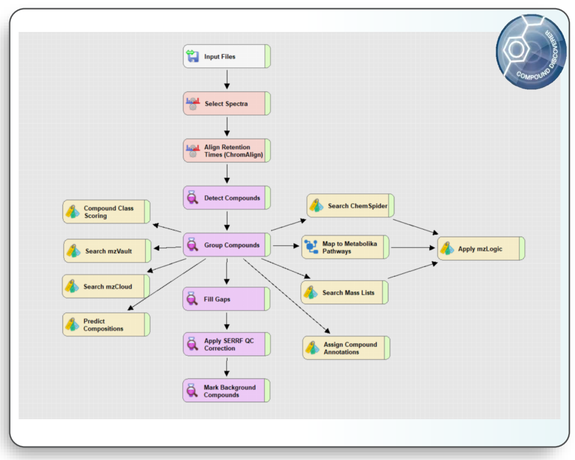


Figure 3. Thermo Scientific™ Vanquish Flex™ UHPLC and Orbitrap IQ-X™ Tribrid™ Mass Spectrometer Methods

Untargeted Analysis



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Targeted Analysis

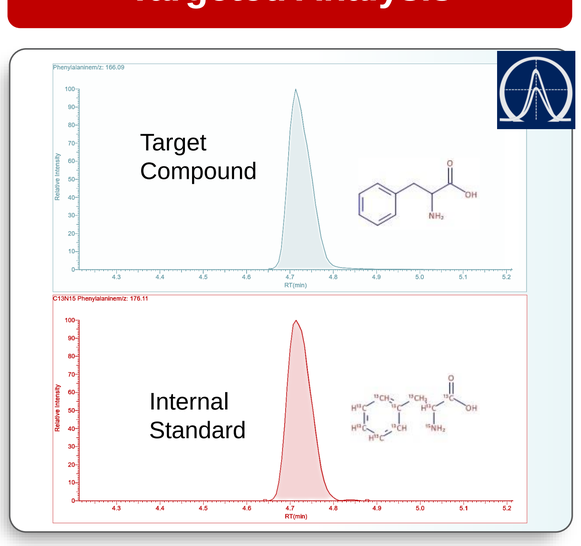
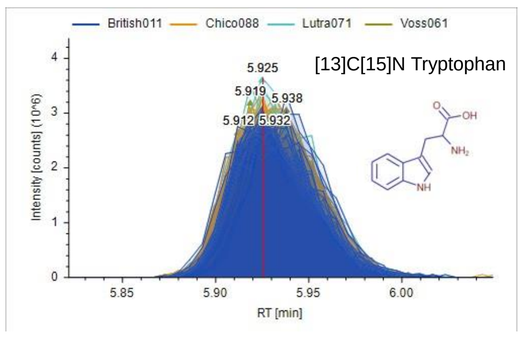


Figure 4. Simultaneous Quantitation and Discovery (SQUAD) Workflow

Results

Quality Control: All samples were spiked with a [13]C[15]N amino acid mix to control for any variability during reconstitution and acquisition. Peak areas for internal standards were consistent across the course of the run and group coefficient of variation (CV) values were 5% or less (Figure 5). Additionally, pooled QC samples were run every 10 samples and used for normalization in Compound Discoverer.



Yeast Strain	Time point	Group CV [%]
British	KO	5
	24 hr	2
	168 hr	3
Voss	KO	3
	24 hr	2
	168 hr	2
Lutra	KO	3
	24 hr	3
	168 hr	5
Chico	KO	5
	24 hr	4
	168 hr	3

Figure 5. Representative internal standard: Extracted ion chromatogram (XIC) and Group CV Values for [13]C[15]N Tryptophan

Multivariate Analysis: The data was subjected to principal component analysis (PCA) to reduce the dimensionality of the data while preserving variability (Figure 6). The results showed that the control and knockout samples had different metabolic profiles than the samples with mid to late dry hopping. PC1 explained 39.8% of the variation while PC2 explained 11.4% (Figure 6a). There was also distinct grouping of yeast strains. British and Chico samples clustered together while Voss and Lutra clustered together. PC1 explained 22.3% of the variation while PC2 explained 13.4% of the variation (Figure 6b).

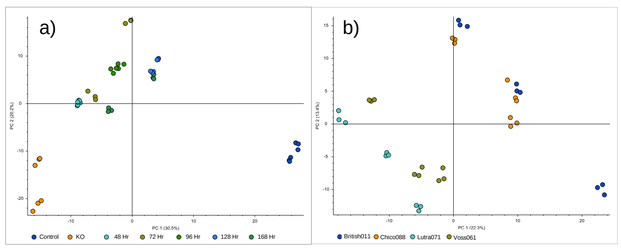


Figure 6. Principle Component Analysis (PCA) showing grouping based on a) hop addition time and b) yeast strain

Data Analysis: Using a SQUAD metabolomics workflow, it was possible to perform accurate quantitation of amino acid compounds and identify unknown metabolites in the same data set. Some of these unknowns included polyphenolic compounds that increased with dry hopping and natural products from hops that contribute to the bitterness and flavor of the beer (Figure 7).

An mzVault library was generated using standards to confidently identify flavonoids which is one of the major compound classes in beer. Analyses revealed that some of the flavonoids showed an increasing trend with dry hop addition time while others remained constant (Figure 8).

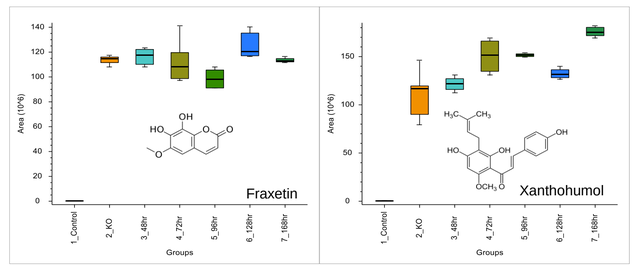


Figure 7. Box and Whisker Plots of Polyphenolic Compounds Identified in Beer

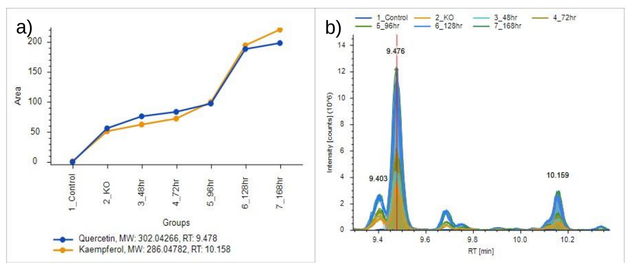


Figure 8. Flavonoids Increasing with Dry Hop Addition Time. a) Trendline Chart b) Chromatographic peaks

Conclusions

This study showed that it is possible to perform targeted quantitation and unknown detection in a single LC-MS injection. Compound Discoverer results showed grouping of specific yeast strains and revealed several compounds that increased with dry hopping. Dry hopping had the most impact on the metabolic changes in the beer samples while yeast strain had less of an impact. Future work includes expanding this study by increasing the number of biological replicates and characterizing the lipids and carbohydrates.

Acknowledgements

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