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

Clara J Myer1, Eric D. Tague1, Christopher Bolcato1, Amirmansoor Hakimi1, Laura Burns2, Lance Shaner2, 1Thermo Fisher Scientific 355 River Oaks Parkway, San Jose, CA 95134, 2Omega Yeast 4720 Pensacola Ave, Chicago, IL 60641

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 distinctive flavors and aromas.

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

Results: A single injection 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).

INTRODUCTION

Beer is a complex beverage made by combining yeast, malt, hops, and water (Figure 1). These are several parameters that can be changed during the brewing process and drastically affect 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 from Omega Yeast to explore the effects of yeast strain and hop addition time on the metabolomics of beer using orbitrap-based LC-MS.

METHODS AND MATERIALS

Sample Preparation: A total of 40 beer samples were collected and stored at -80°C 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.

LC-MS Methods: All samples were reconstituted in 300 µl of 50/50 MeOH:H2O containing 500 nM of 13C15N amino acid mix and then subjected to LC-MS analysis. Metabolites were separated on a Hypersil GOLD™ (150 x 2.1 mm, 1.9 µm) column using a neat India Pale Ale and analyzed using an Orbitrap Q-X™ Tribrid™ mass spectrometer. All samples were run in full scan using high resolution (i.e., 120k) and then pooled to collect data-dependent MS2 spectra using Acquity (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).

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 hoppy samples had different metabolic profiles than the samples with mid to late hop addition. PC1 explained 80% of the variation while PC2 explained 11% (Figure 6A). There was also distinct grouping of yeast strains. Brown and Chico samples clustered together while Vox and Lutra clustered together (Figure 6B). PC1 explained 32.3% of the variation while PC2 explained 13.4% of the variation (Figure 6B).

RESULTS

Quality Control: All samples were spiked with a [13C15N] amino acid mix to control for any variability during recombinant 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.

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

I would like to thank Brandon Bills from the product management team for allowing me to use his IQ-X and lab space to conduct this project and Bashar Amer for assistance with sample preparation equipment in the vertical marketing lab.

TRADEMARKS/LICENSES

© 2023 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries. This information is not intended as an endorsement or as an encouragement of the use of these products in any manner that might infringe the intellectual property rights of others.