Beer Processomics:

Targeted and untargeted metabolomics of a SMaSH beer reveals the molecular evolution of volatile and nonvolatile metabolites throughout brewing

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ASBC
THE SCIENCE OF BEER

BREWING SUMMIT 2022
Providence, Rhode Island | August 14–16

Overview

BACKGROUND

- Targeted vs untargeted metabolomics

EXPERIMENTAL DESIGN

- Analytical workflow
- Brewing
- Final beer

TARGETED METABOLOMICS

- Amino acid metabolism
- Evolution of flavor cmpds
- Evolution of hop cmpds

UNTARGETED METABOLOMICS

- Quality control
- Global analysis

Targeted vs **untargeted** metabolomics

Targeted

- Hypothesis-driven
- Subset analysis
- Correlated to reference standards
- Identification known
- Absolute quantitation

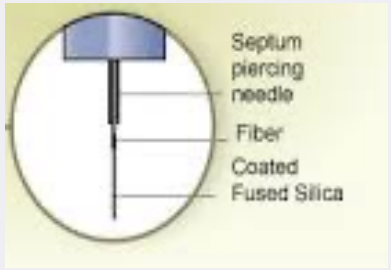
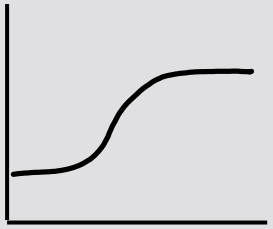
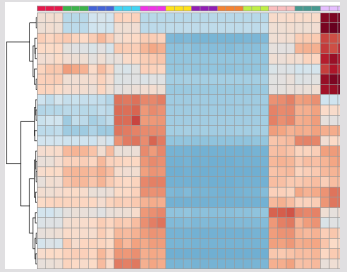
Untargeted

- Hypothesis-generating
- Global analysis
- Correlated to databases/libraries
- Qualitative identification
- Relative quantitation

iStock photos

Experimental Design

Analytical workflow

	Sample Prep	Separate & Detect	Identify	Differential Analysis
GC-MS *Volatile Flavor Cmpds*	SPME 	DB5-MS Single quad GC/MS	NIST database + stds	Targeted 
LC-MS *Nonvolatile Cmpds*	Sonicate, filter & dilute	**QqQ** (targeted) **q-TOF** (targeted & untargeted	stds, homebuilt hops DB, Metlin, KEGG	Untargeted 

MS workflow

		GC/MS	RP LC	HILIC LC
Targeted		Flavor compounds	**QqQ** Hop compounds	**q-TOF** Amino acid metabolism
Untargeted			**q-TOF** (+) and (-) ESI w/ int std	**q-TOF** (+) ESI w/ int std

SMaSH pale ale

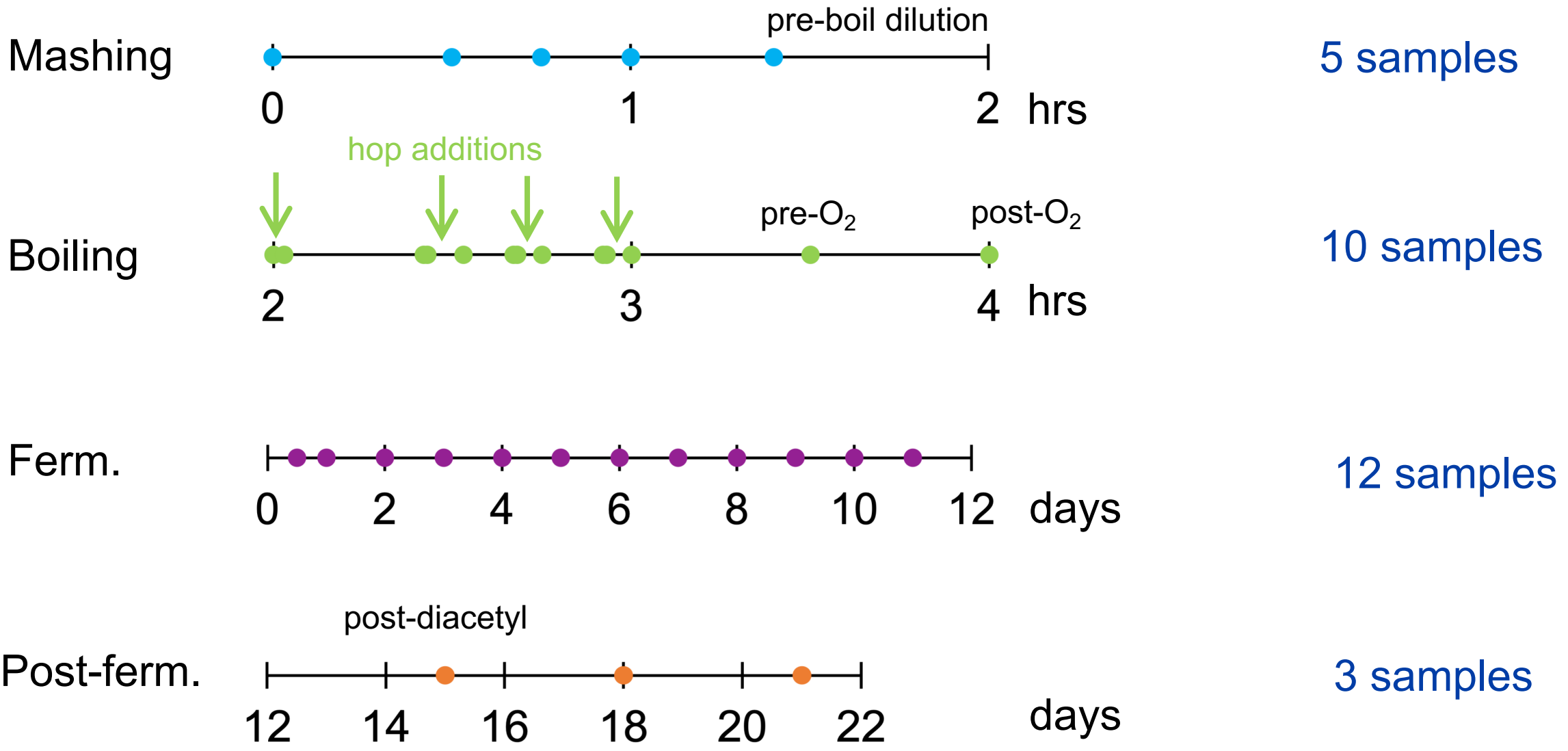
2-row malt

Citra hops

001 California ale yeast

SS Brewtech 1-barrel brewhouse & fermenter

Beer processomics sample collection



SMaSH beer characterization

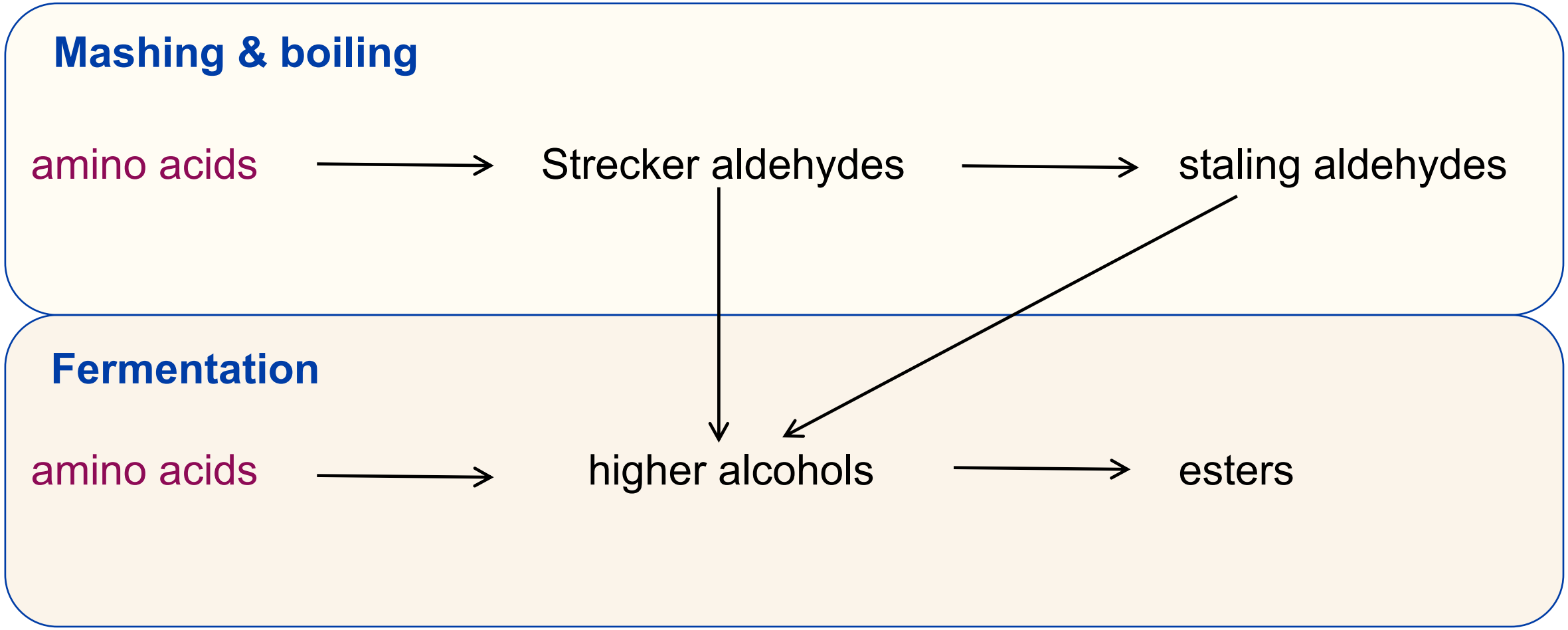
Instrumental Analysis students characterized the final beer using ASBC methods.

Color (ºSRM)	4.9 ± 0.5 (n = 7)
Total Phenols (mg/L)	308 (n = 1)
IBUs (out of 100)	61.5 ± 12.0 (n = 7)
% Protein (%wt/wt)	0.671 ± 0.114 (n = 6)
% ABV (GC-FID)	7.4% (n = 1)

Image from: https://brookstonbeerbulletin.com/thinking-about-beer-color/

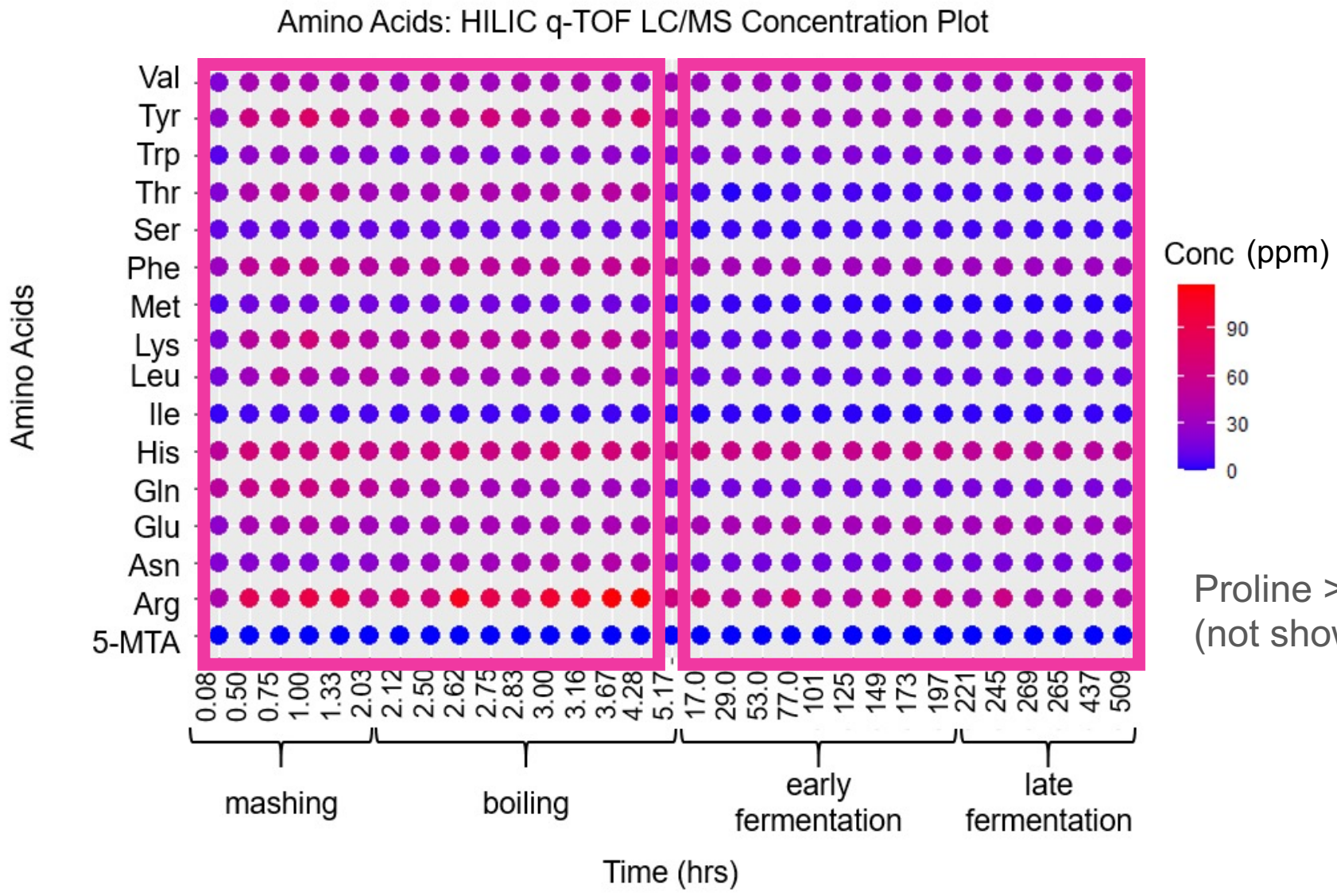
Targeted metabolomics

Targeted: Amino acid metabolism → flavors



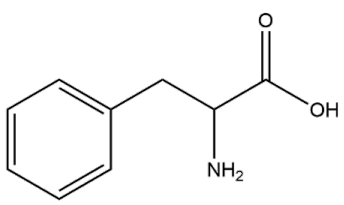
Adapted from Ferreira, I.M., Guido, L.F. *Fermentation,* **2018**, *4*, 23, doi:10/3390/fermentation4020023

Targeted: Amino acid metabolism

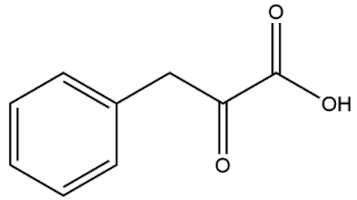


Proline > 200 ppm (not shown)

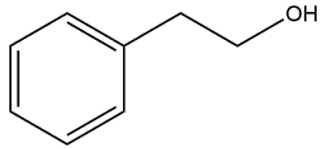
Targeted: Phenylalanine in Ehrlich pathway



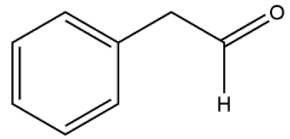
phenylalanine
HILIC LC/MS



phenylpyruvate
RP (-)ESI LC/MS



2-phenylethanol
GC/MS



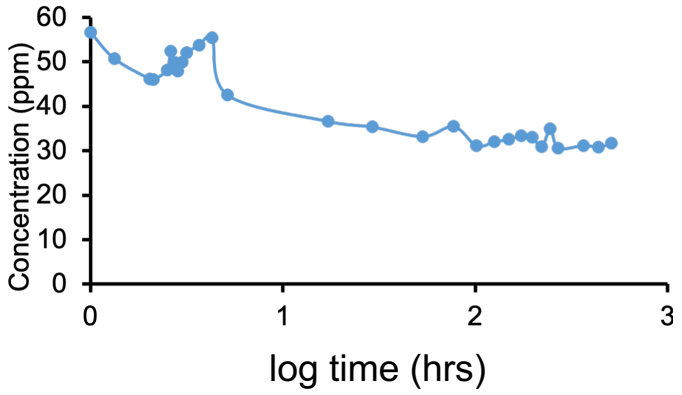
phenylacetaldehyde
GC/MS

Confirmed w/ std

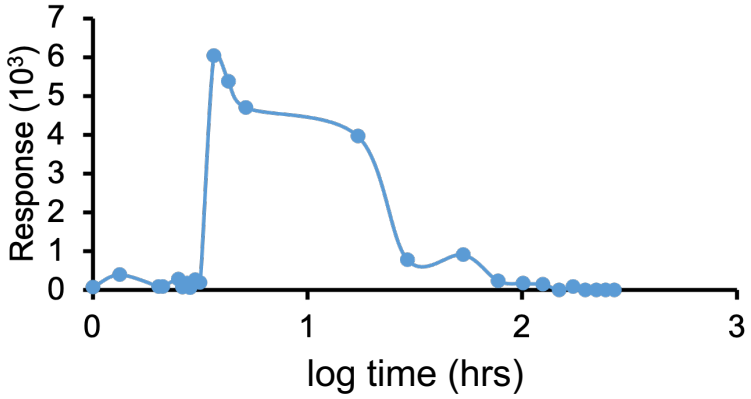
Putative ID

Targeted: Phenylalanine in Ehrlich pathway

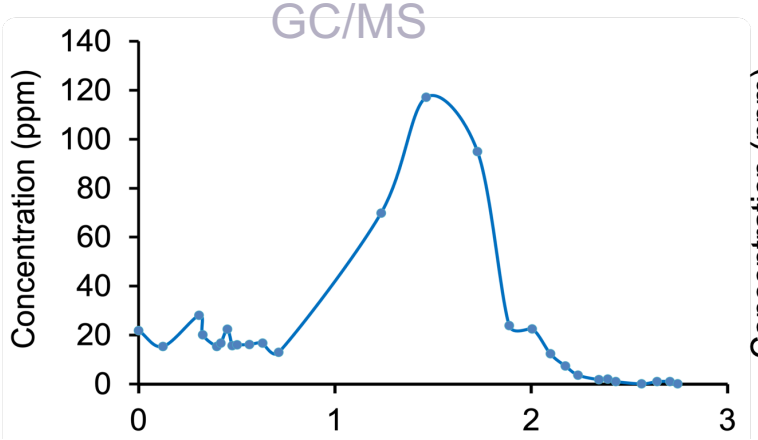
phenylalanine
LC/MS



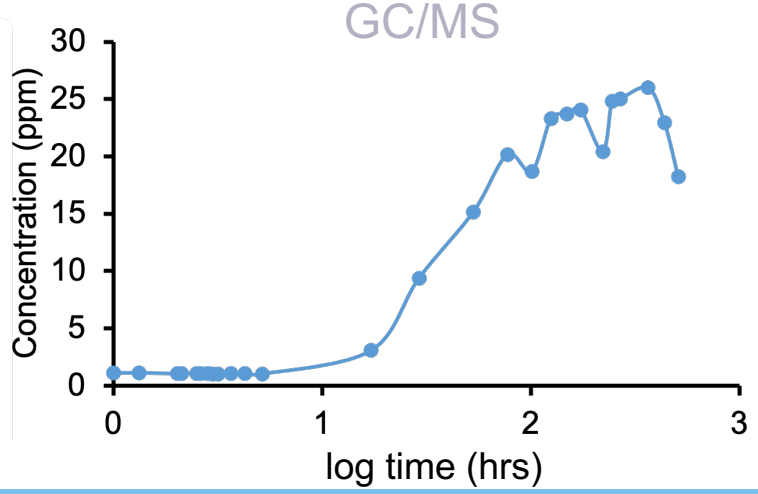
phenylpyruvate
LC/MS



phenylacetaldehyde
GC/MS



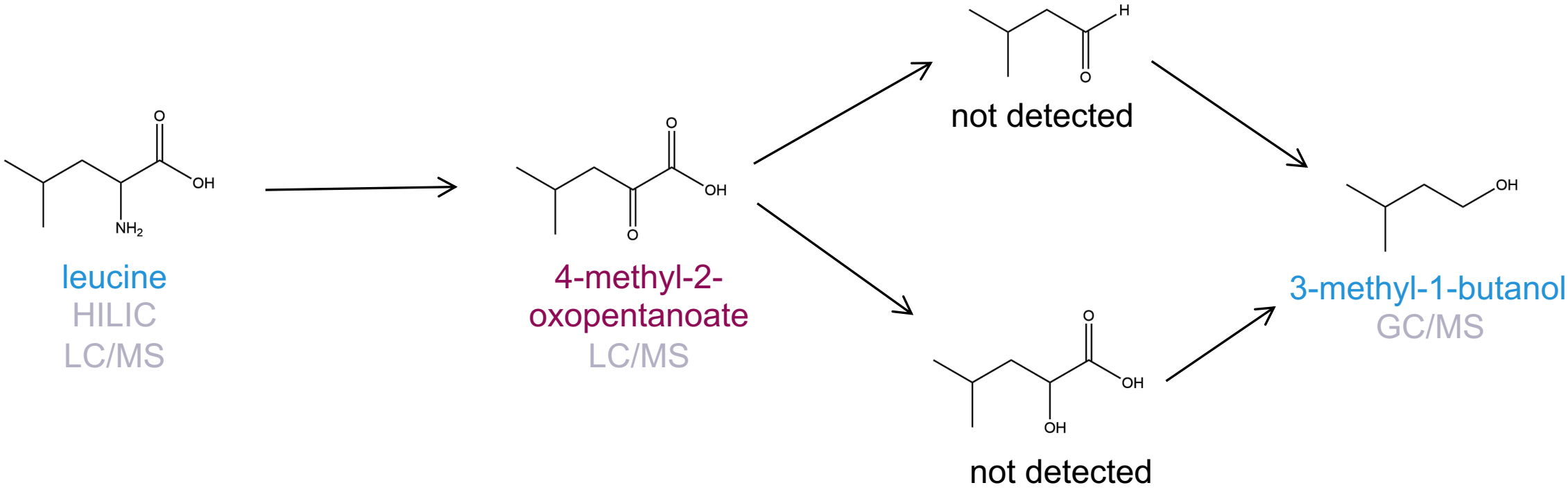
2-phenylethanol
GC/MS



Confirmed w/ std

Putative ID

Targeted: Leucine in Ehrlich pathway



Confirmed w/ std

Putative ID

Targeted: Leucine in Ehrlich pathway

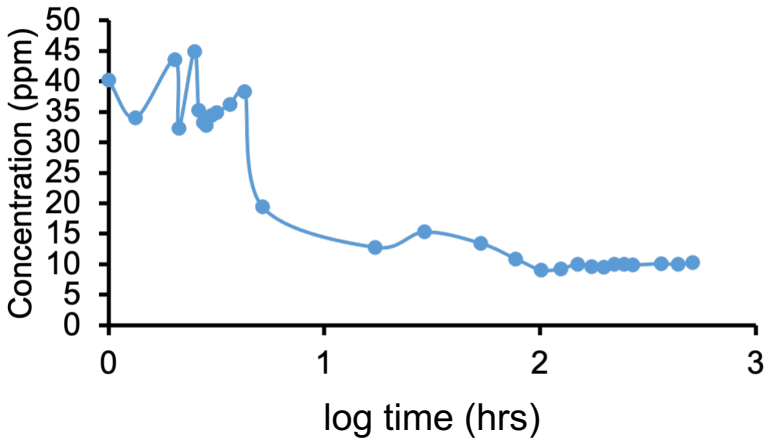
leucine
LC/MS

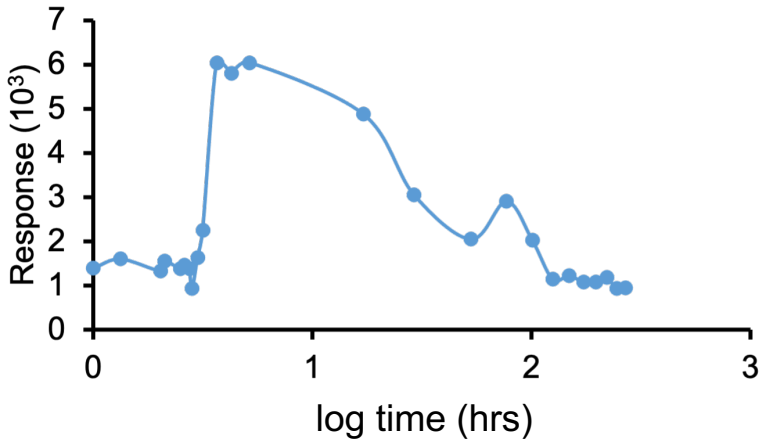
→

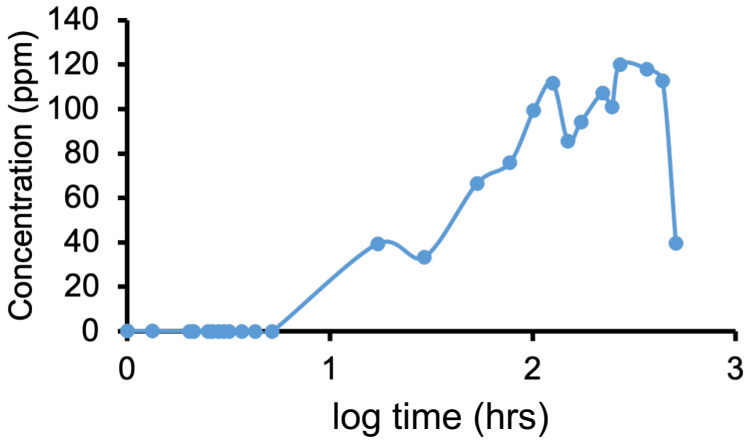
4-methyl-2-oxopentanoate
LC/MS

→

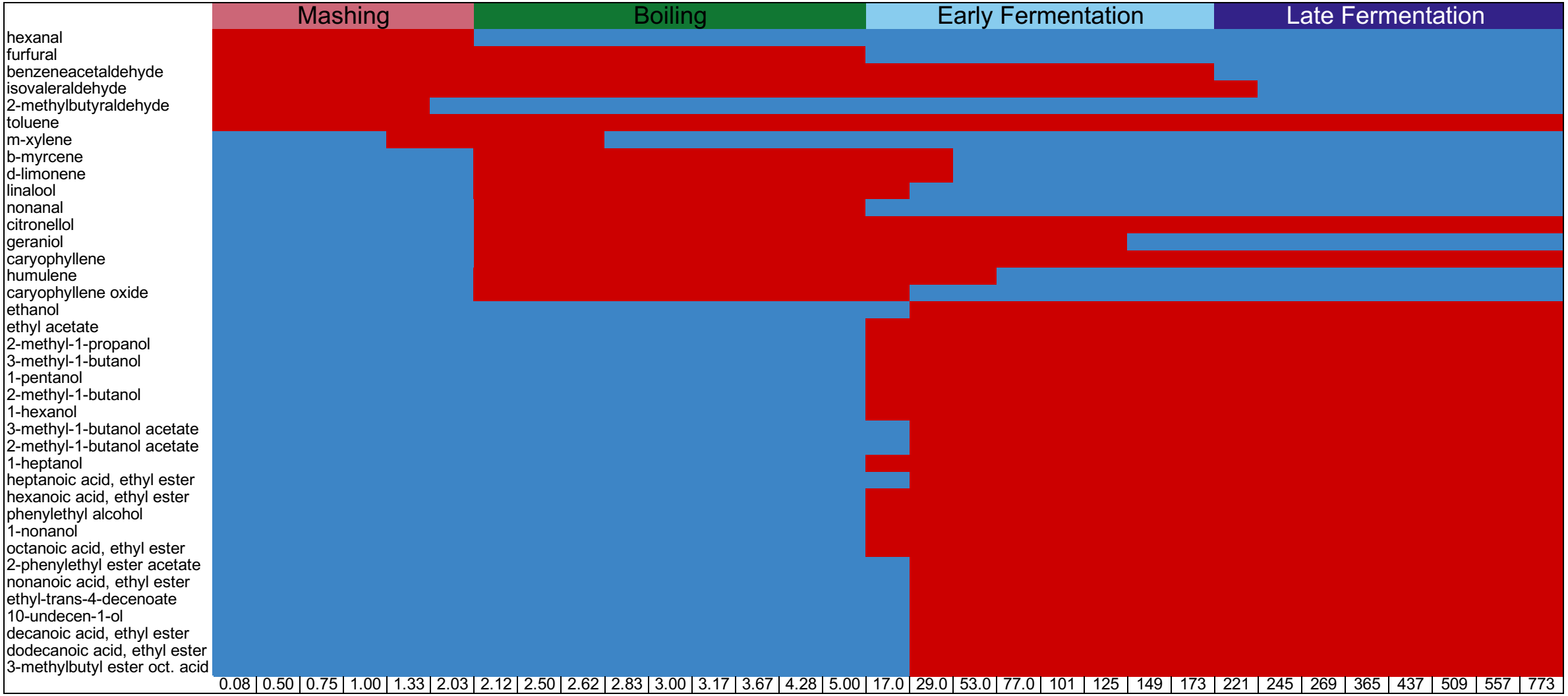
3-methyl-1-butanol
GC/MS





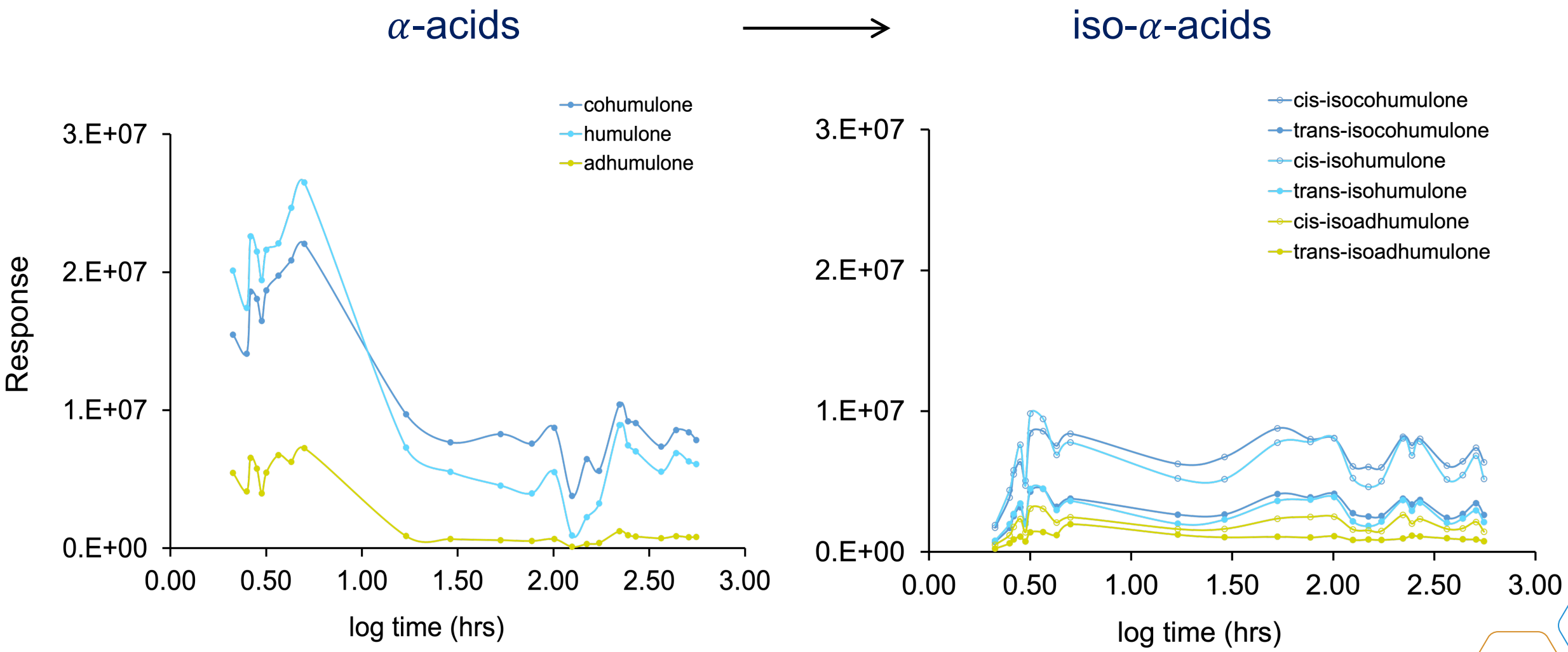


Targeted: Other flavor compounds

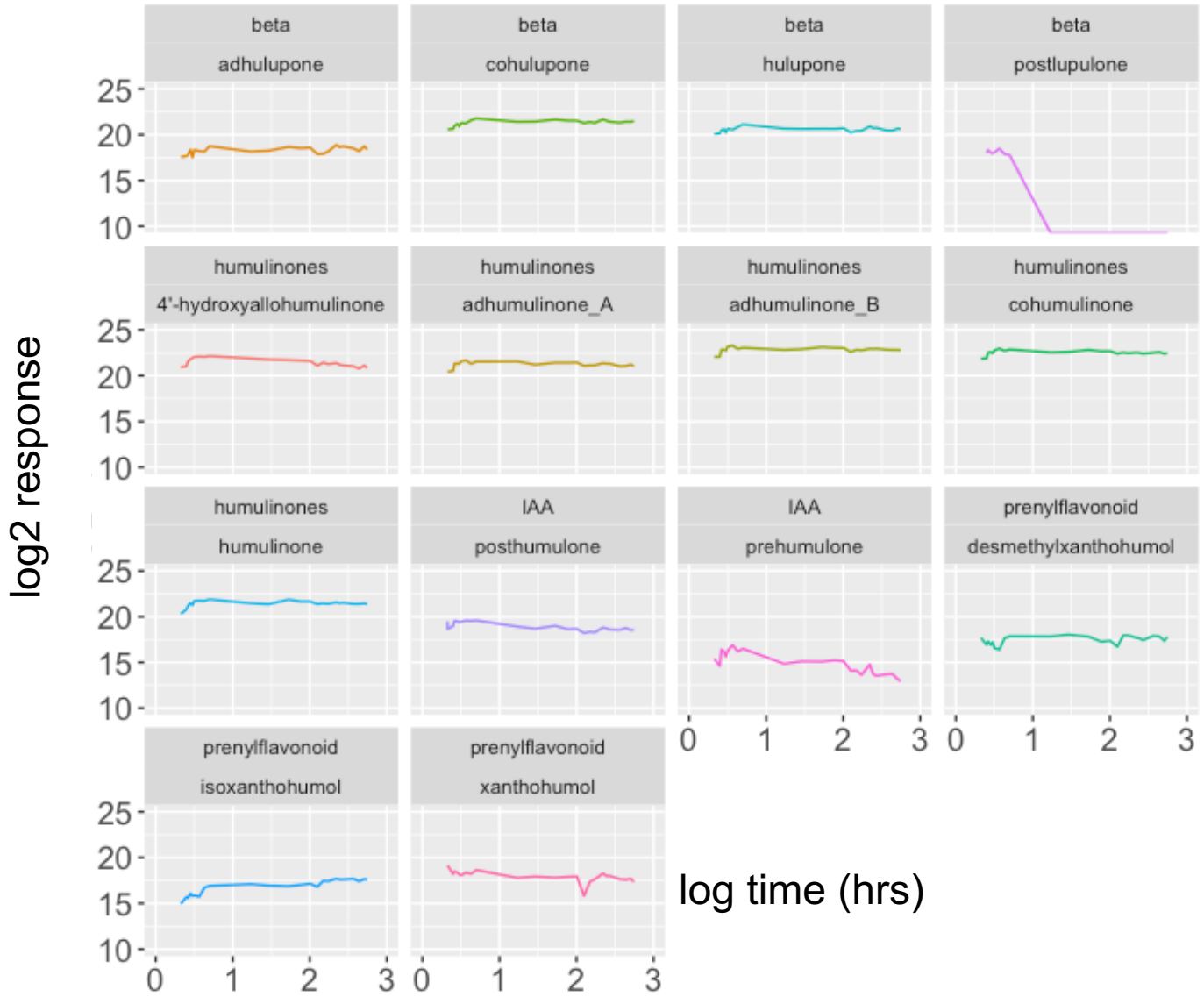


Brewing Hrs

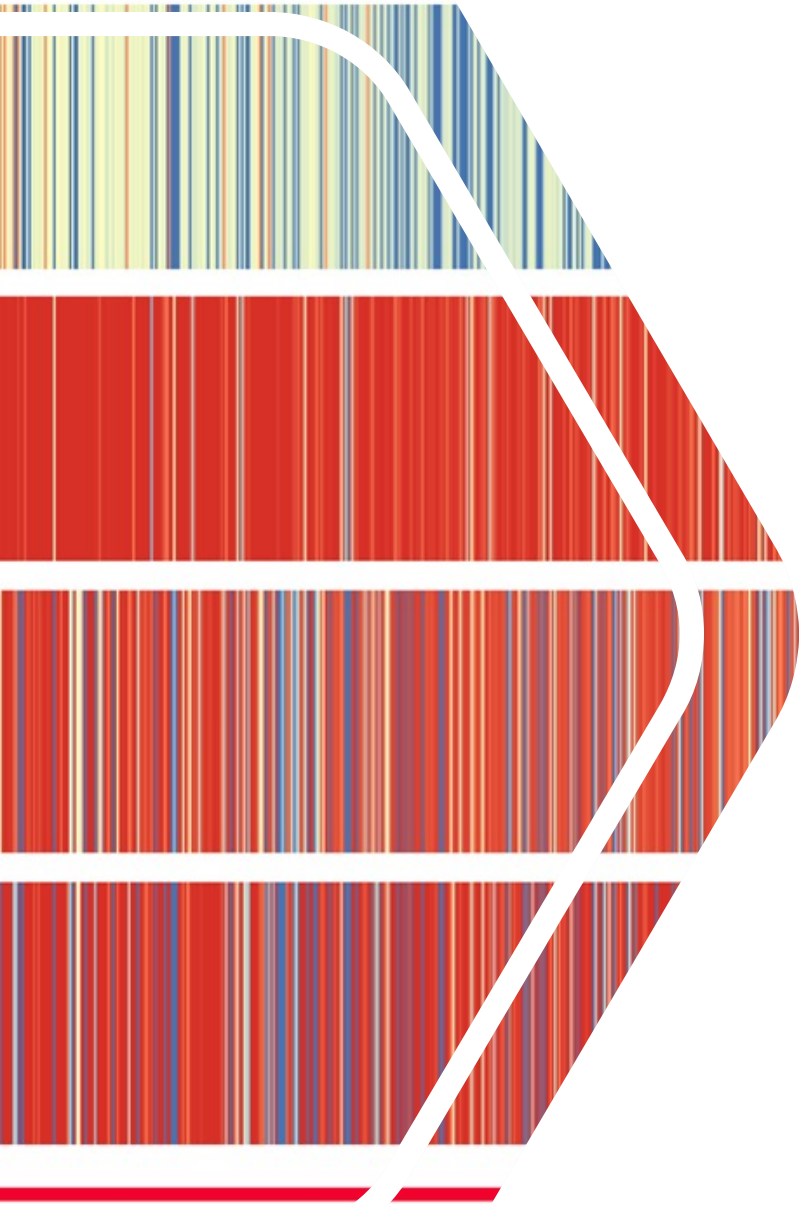
Targeted: Hop compounds (LC/MS)



Targeted: Hop compounds* (LC/MS)



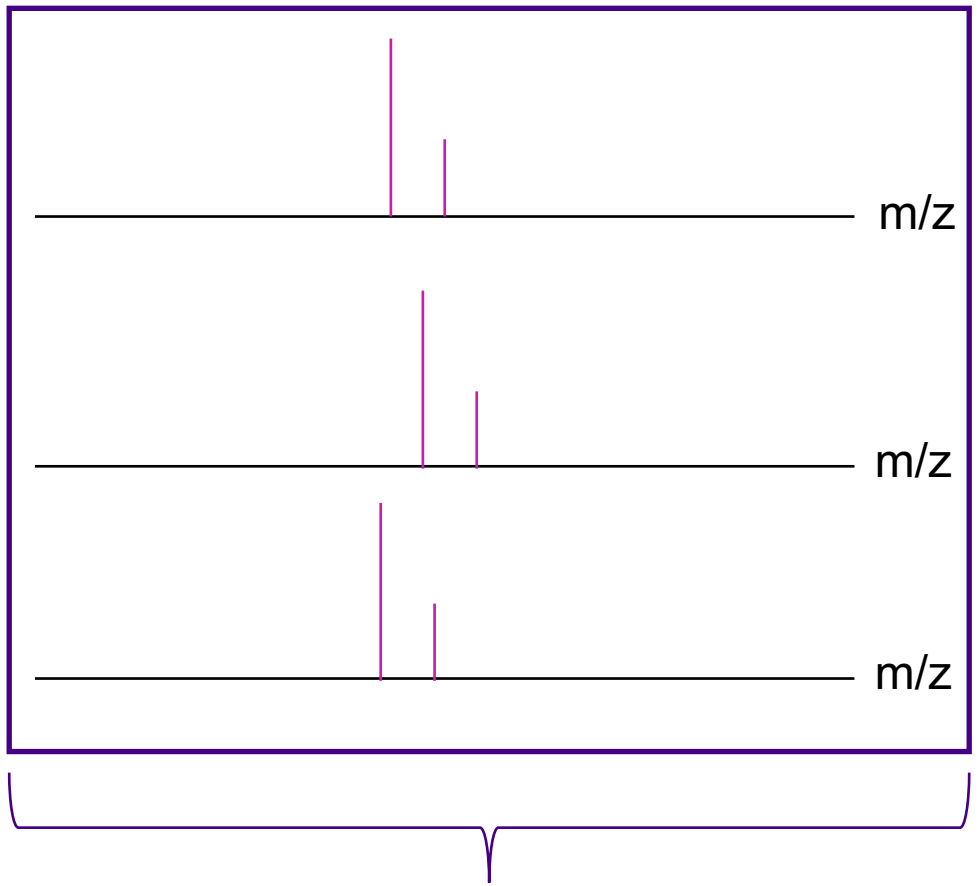
**putative IDs from matching MS/MS spectra to the literature*



Untargeted metabolomics

Untargeted: QC with int. stds

Molecular Feature (MF) = Unique mass & RT



X 1000s MFs

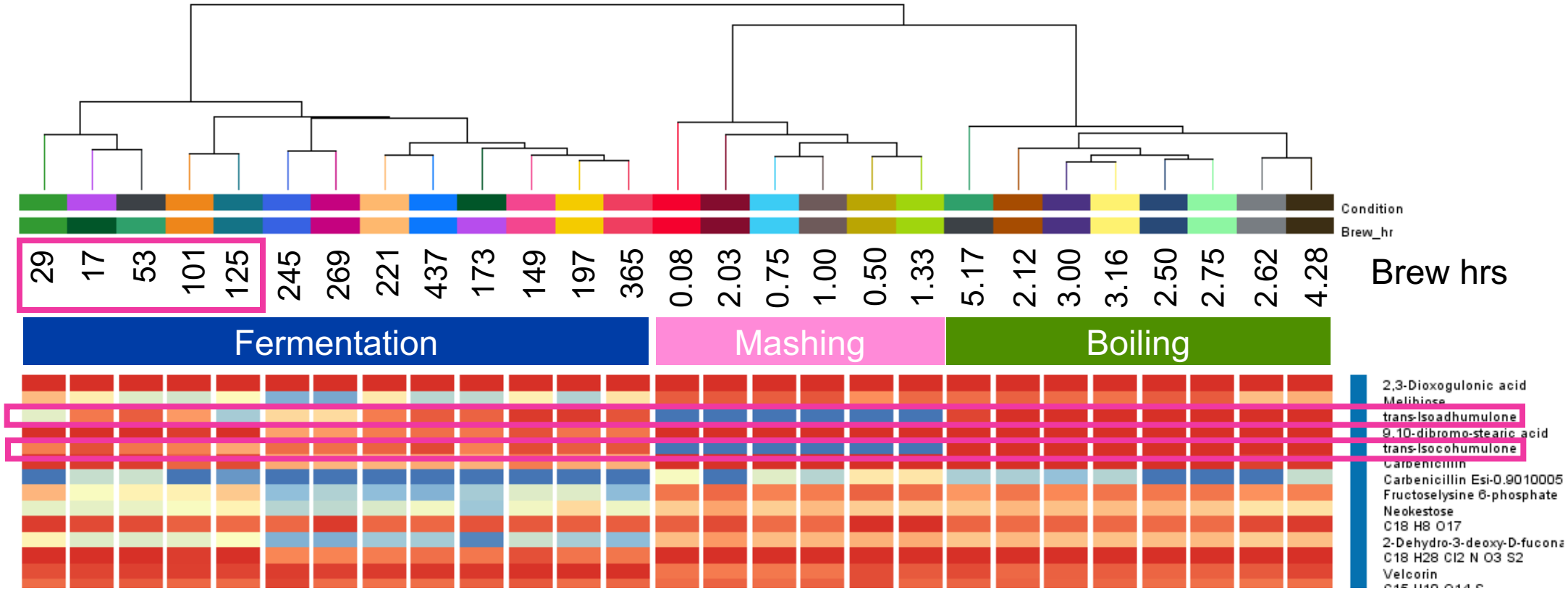
RT window = 0.15 min.
m/z window = 15 ppm

Untargeted: QC with int. stds

RP LC (-) ESI q-TOF MS

8 data collection days	internal std = 1-naphthoic acid
RT, std dev (min)	0.011
RT, %RSD	0.15%
Response, %RSD	7.54%
Avg mass error, ppm	< 2 ppm

Untargeted: (-)ESI LC/MS



+ 1500 other molecular features

Conclusions & Future Work

Targeted

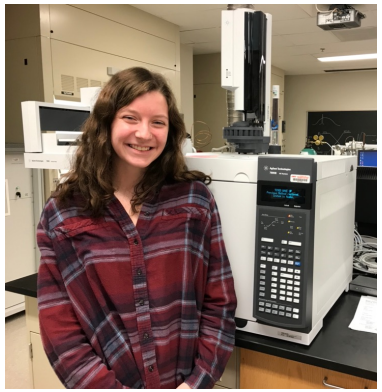
- Built a robust mass spec-based beer processomics platform that allows monitoring of volatile and nonvolatile compounds by GC/MS and LC/MS, respectively
- Targeted metabolomics focused on metabolic pathways that produce flavor compounds from amino acids
- Need to confirm intermediate metabolites by matching to standards

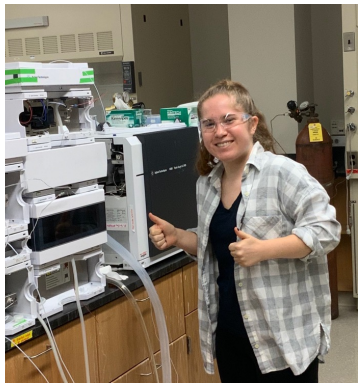
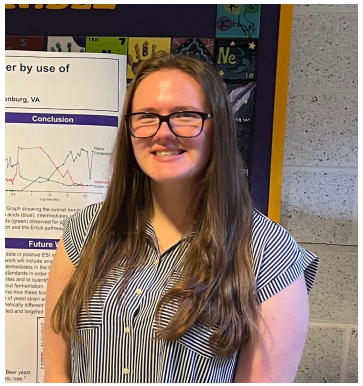
Untargeted

- Established quality control metrics to ensure the best molecular feature alignment across RP and HILIC q-TOF LC/MS data in both positive & negative ion ESI modes to afford the greatest metabolite coverage for new compound discovery.

Summer 2022 fermented a SMaSH wort with 5 genetically different yeast.

Acknowledgements



Agilent Technologies

American Society for Mass Spectrometry PUI Research Award

Department of Chemistry & Biochemistry

NSR Research Experience for Undergraduates (REU) CHE - 1757874