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Underivatized Amino Acid Profiling in Fermentation Samples with Novel High-Resolution Mass Spectrometry Platform with Bio-Compatible Column and LC

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Introduction

Bio-compatible LC and inert column for LC/MS analysis of amino acids during yeast fermentation

Saccharomyces cerevisiae, commonly known as Baker's Yeast, has been used for centuries in a range of food processes, including the production of bread, wine, and beer. The fermentation process of yeast converts sugars from their environment to alcohol and organic acids and provides essential energy. Yeast grow across wide environmental niches - intaking amino acids, vitamins, minerals, and sugars from their surroundings to support growth and fermentation.[1]

Monitoring of amino acid uptake can be performed using liquid chromatography – mass spectrometry (LC/MS) and provide information on yeast fermentation and growth performance. In this study, the uptake of amino acids throughout yeast fermentation is quantified using a bio-inert Altura column[2] and bio-compatible LC coupled to a novel high-resolution mass spectrometer, the Agilent 6230C TOF (Fig. 1).

Figure 1. The workflow utilizes chromatographic separation over an Altura HILIC bio-inert column using an Agilent 1290 Infinity II Bio LC and the Agilent 6230C TOF instrument.

Traditional workflows for amino acid analysis and quantitation often utilize derivatization to improve column retention or ionization. Here, a previously described HILIC methodology to chromatographically separate underivatized amino acids is utilized to quantify concentration changes over time within matrix.[3] We demonstrate robust detection and quantitation of amino acids across a wide dynamic range within a complex metabolite-rich sample matrix. This workflow can be applied to the monitoring of yeast fermentation in food processes and similar applications.

Experimental

Preparation of amino acids derived from yeast fermentation media

Yeast (ATCC 204508) was grown in dilute Yeast Peptone Dextrose broth (Teknova Y5000) at 30°C. Discrete time points at two-hour increments between 0 and 12 hrs. and a 24-hr. time point were taken. Yeast were centrifuged at 5,000 x rcf for 5 min., and the supernatant was collected. Amino acids were extracted via dilution of the supernatant 1:4 with 50% MeOH:H_20 and centrifugation at 10,000 x rcf for ten minutes and the supernatant harvested.

Yeast was diluted 20x in 70:20:10 ACN:H_20:MeOH and injected onto the LC/TOF for analysis. A standard curve was generated using Agilent Amino Acid standards (5061-3330), with glutamine, asparagine and tryptophan spiked in.

Table 1. LC conditions for the amino acid analysis.

LC Conditions			
Column	Agilent Altura Poroshell HILIC-Z, 2.1x150mm, 2.7µm PN: 227215-924		
Column temp.	30°C		
Autosampler temp.	4°C	**Injection volume**	3 µL
Needle wash	Multiwash: 3s each: IPA, water, ACN		
Mobile phase	**A:** 10mM ammonium acetate, pH 9.0 **B:** 10 mM ammonium acetate, pH 9.0, in 90:10 ACN:H_20		
Flow rate	0.25 mL/min		
Gradient program	0 min 2 min 12 min 13 min 16 min 17 min 25 min		90% B 90% B 40% B 20% B 20% B 90% B 90% B
Total run time	25 min		

Table 2. MS conditions for the amino acid analysis.

6230C MS Conditions	
***m/z* range**	60-1000
Sheath Gas Temperature	300 ºC
Sheath Gas Flow	12.0 L/min
Gas Temperature	200 ºC
Gas Flow	10.0 L/min
Nebulizer	40.0 psi
Capillary	3000 V (-)
Nozzle Voltage	0 V (-)

Results and Discussion

HILIC chromatography of amino acids over an Altura column shows good LC peak separation

Amino acid standards (n=4) ranging from 50 – 50,000 nM were injected onto the bio-inert Altura Poroshell HILIC-Z column with 3 µL injection volume (Fig. 2). The Altura demonstrates injection-to-injection reproducibility (Fig. 3).

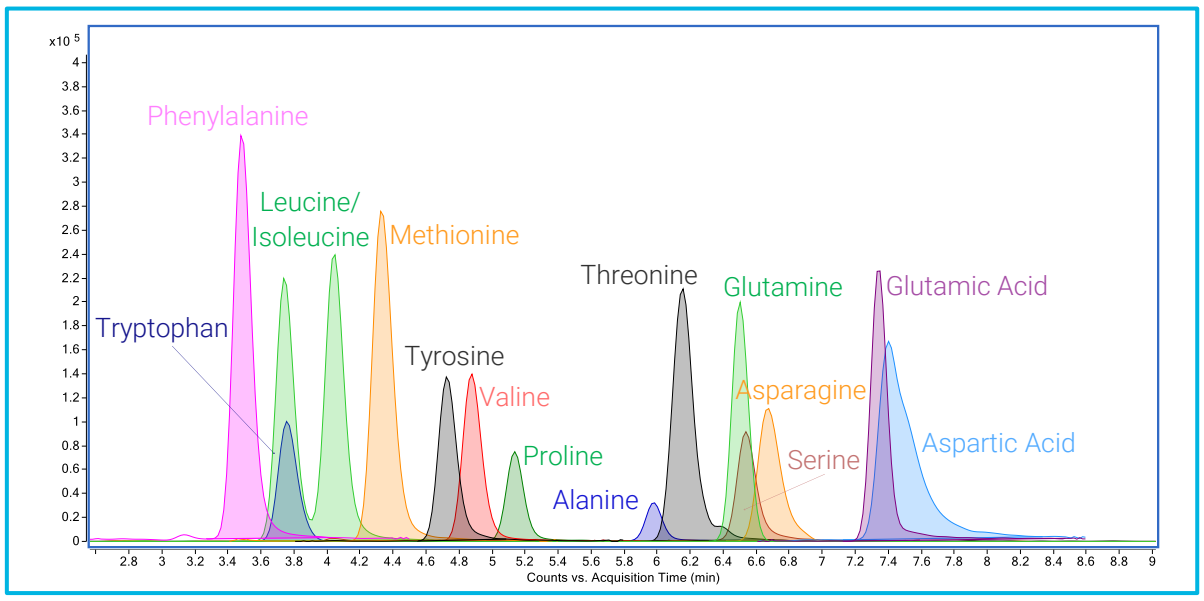

Figure 2. Extracted ion chromatogram (EIC) traces of amino acids within the gradient at 2.5 µM standard concentration.

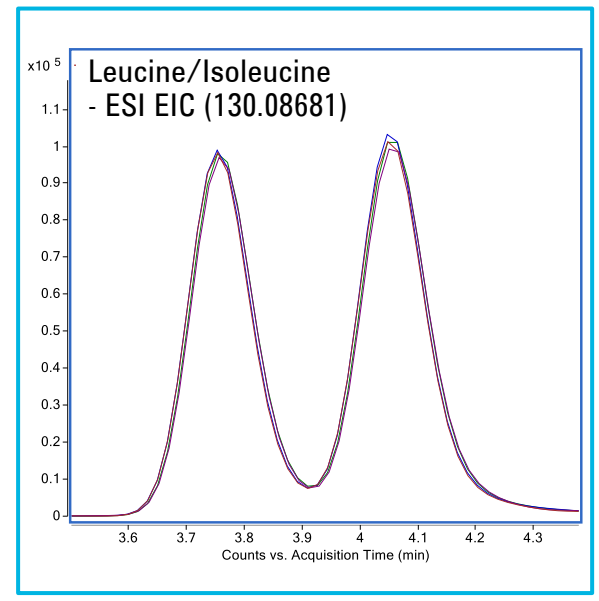

Figure 3. EIC trace of leucine and isoleucine (*m/z 130.0868*) from four replicate injections of 1 µM standard (3 µL), showing separation of isomers and peak reproducibility between the injections.

Quantitation of amino acids over a wide dynamic range allows for application in complex matrices

To quantify the amino acids in matrix, a retention time library was generated using the amino acid standards. Calibration curves were created based on estimates of the amino acid concentration in the samples (Fig. 4, Table 3).

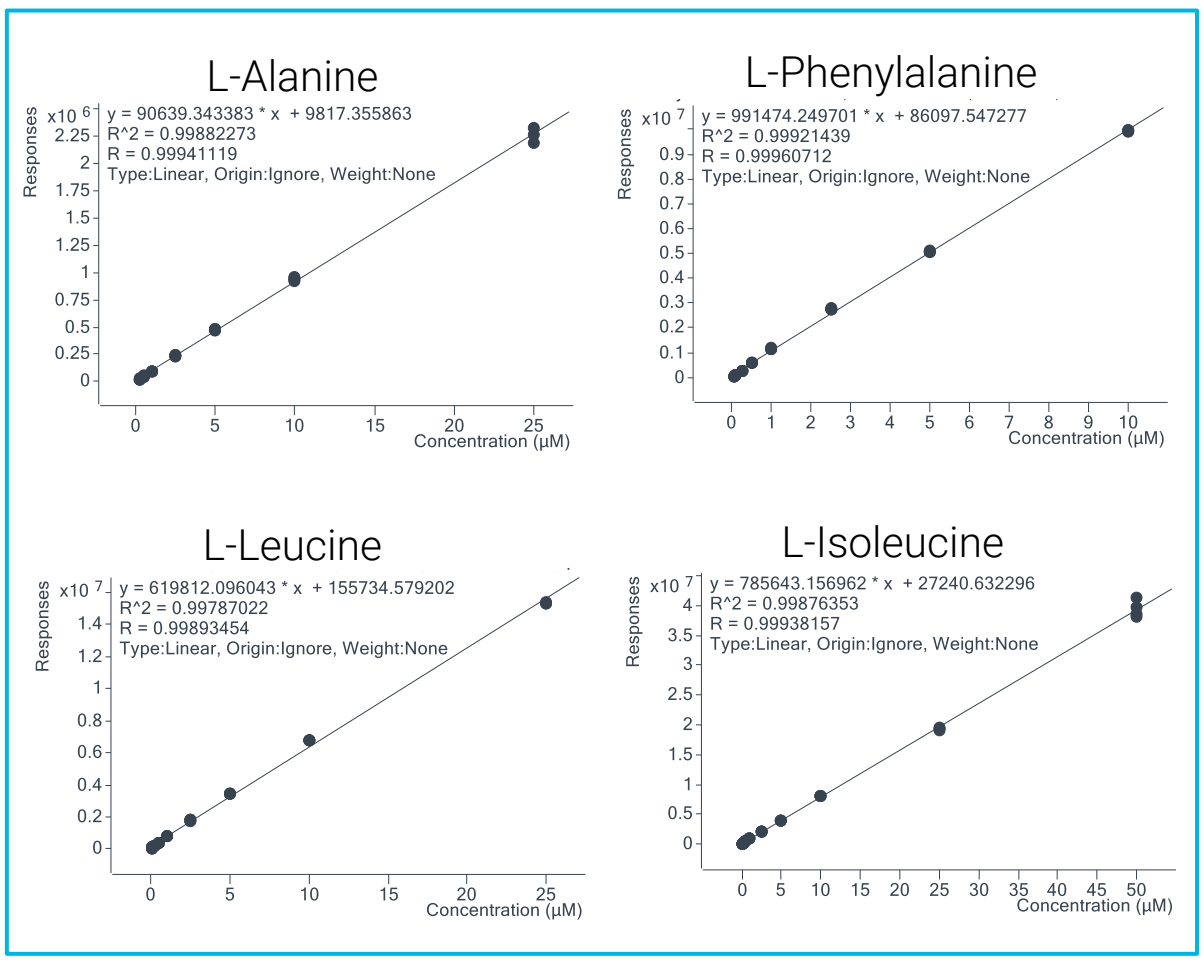

Figure 4. MassHunter Quant 12.2 analysis generates calibration curve-at-a-glance.

Table 3. Summary of the quantitative results for the amino acid standards in negative ionization mode. * indicates best fit as quadratic.

Compound	RT	Cal Curve range (nM)	R^2
Alanine	6.0	250 – 25,000	0.999
Asparagine	6.7	100 – 10,000	0.999
Aspartic Acid	7.4	100 – 10,000	0.998
Isoleucine	4.1	50 – 50,000	0.999
Glutamic Acid	7.3	100 – 10,000*	0.999
Glutamine	6.5	250 – 25,000*	0.999
Glycine	6.5	250 – 5,000	0.998
Leucine	3.7	50 – 25,000	0.998
Methionine	4.4	50 – 10,000	0.999
Serine	6.5	250 – 25,000*	0.998
Phenylalanine	3.5	50 – 10,000	0.999
Proline	5.1	100 – 50,000	0.999
Threonine	6.2	100 – 10,000*	0.999
Tryptophan	3.8	250 – 10,000	0.999
Tyrosine	4.7	50 – 10,000	0.999
Valine	4.9	50 – 50,000	0.998

Calibration curve shows high dynamic range and linearity

Calibration curves were created using amino acids at a wide range of concentrations (50 – 50,000 nM). For many amino acids, a dynamic range of up to a thousand-fold change in concentration could be analyzed with high linearity and reproducibility (R^2 > 0.998 & RSDs < 10%) (Fig. 4, Table 3). The 6230C obtains high mass accuracy for these compounds with <2 ppm deviation. These curves cover the broad distribution in amino acid concentrations found within the sample matrix.

Results and Discussion

Quantitation of yeast amino acids reveal differential uptake of metabolites during the yeast fermentation process

During fermentation, yeast uptake sugars, vitamins, minerals, and amino acids from their environment and release metabolites. Amino acid levels can be quantified over time to track uptake and release using MassHunter Quant 12.2 (Fig. 5, Table 4) while untargeted analysis with MassHunter Explorer 2.0 can track overall changes in features (Fig. 6).

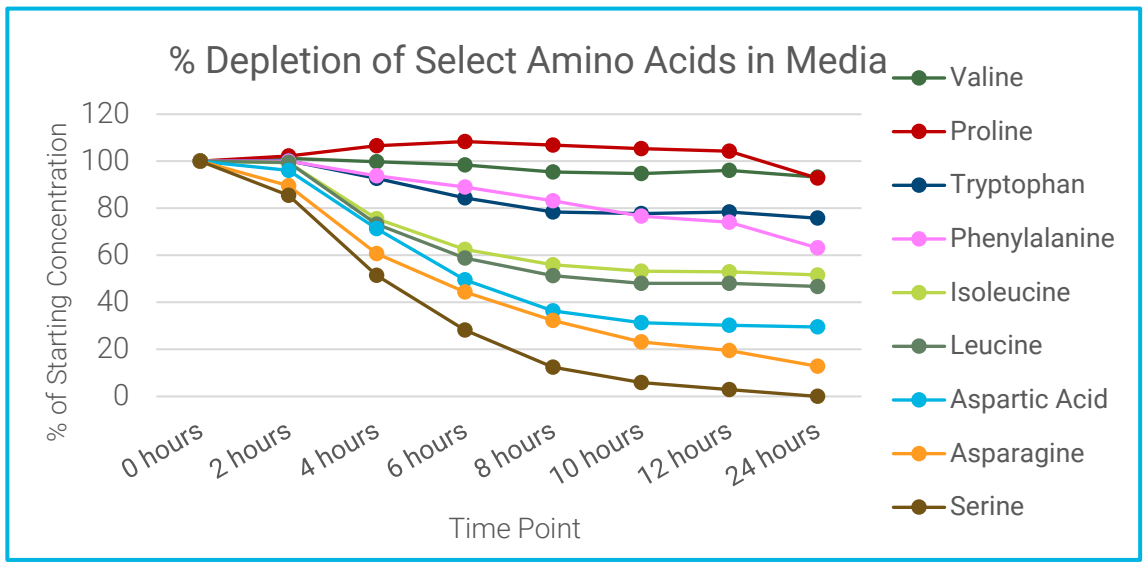

Figure 5. Depletion of amino acids within the growth media at discrete time points (mean, n=6).

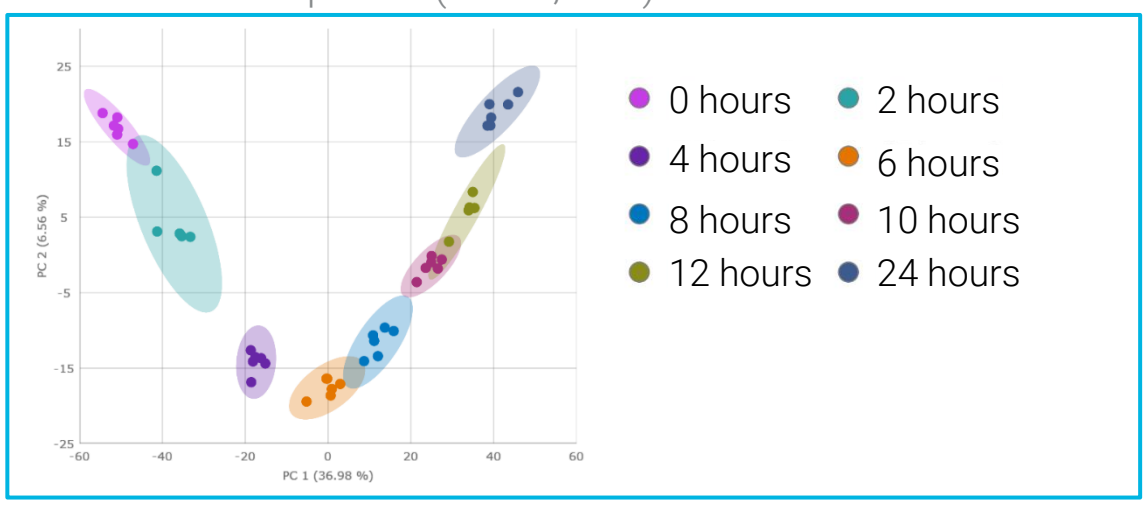

Figure 6. Principal Component Analysis (PCA) of overall features over time using MassHunter Explorer 2.0.

Additional fermentation byproducts can be monitored

Fermentation processes sugars to be broken down into organic acids and alcohol. Media hexose concentration as a function of time can be monitored (Fig. 7) High pH mobile phase favors deprotonation of amino acids for negative ionization detection and simultaneous detection of charged organic acids that are a fermentation byproduct (Fig. 8).

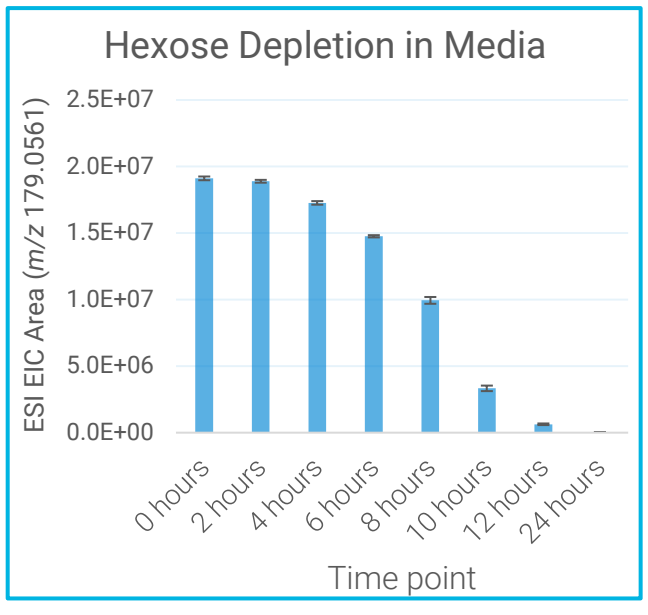

Figure 7. Decrease in available hexose in media over time. Data is represented as mean ± SD , n=6.

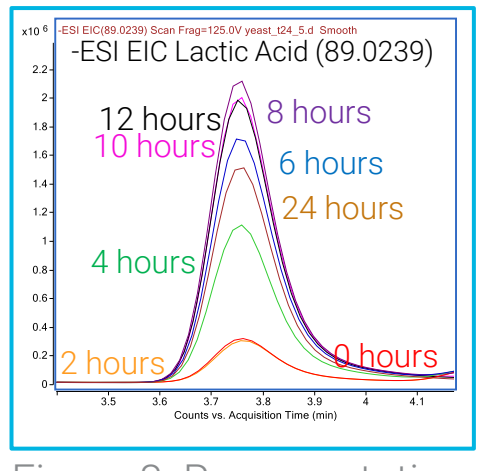

Figure 8. Representative traces of lactic acid from each time point.

Table 4. Quantification of the initial amino acids in the media. Supernatant was diluted 20x prior to injection.

Amino Acid	Starting Concentration (µM) (mean ± SD)	Amino Acid	Starting Concentration (µM) (mean ± SD)
Alanine	135.6 ± 4.4	Methionine	1.5 ± 0.2
Asparagine	24.0 ± 0.4	Serine	32.7 ± 1.6
Aspartic Acid	28.0 ± 0.6	Phenylalanine	31.3 ± 0.5
Isoleucine	53.1 ± 0.9	Proline	16.9 ± 0.3
Glutamic Acid	71.4 ± 2.3	Threonine	27.0 ± 0.8
Glutamine	4.8 ± 0.1	Tryptophan	8.4 ± 0.3
Glycine	1.7 ± 0.2	Tyrosine	9.0 ± 0.4
Leucine	63.3 ± 1.1	Valine	39.1 ± 1.0

Conclusions

Quantitative workflow for the analysis of amino acids in matrix using bio-inert Altura column and novel TOF

- Altura column and Bio LC demonstrate good peak retention and shape while novel 6230C TOF shows high resolution and coverage over an extended dynamic range of analyte concentration
- High pH mobile phase and negative mode detection allows for easy simultaneous monitoring of amino acids, sugars, and organic acid fermentation byproducts

References

[1]Maicas, Sergi. "The Role of Yeasts in Fermentation Processes." *Microorganisms* vol. 8,8 1142. 28 Jul. 2020, doi:10.3390/microorganisms8081142

[2]Hsiao, J. et al. Pushing the Boundaries of Chromatographic Separation with Inert HPLC Column Hardware. Agilent White Paper 5994-8618EN. 2025.

[3]Hsiao J. et al. Analysis of Underivatized Amino Acids by LC/MS for Bioreactor Cell Culture Monitoring. Agilent Application Note 5991-8816EN. 2018.

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