96 Bottles of Beer: Metabolic Profiling of Spent Growth Media Using Rapid, High Throughput Capillary Electrophoresis-Electrospray Ionization-Mass Spectrometry

Joshua P. Guerrette, Erin A. Redman, J. Scott Mellors

908 Devices Inc., Boston, MA and Carrboro, NC

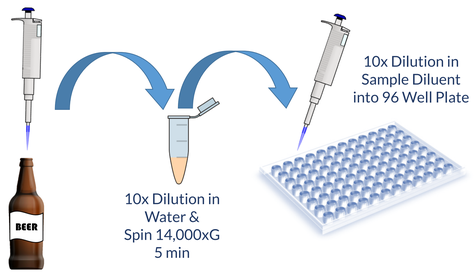
Overview

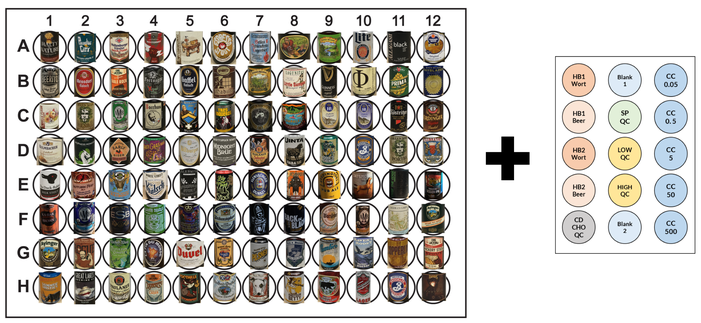
Bioreactor monitoring is a vital part of quality control and process refinement. There is an ever-growing demand for high throughput analysis methods capable of delivering metabolic profiling data rapidly to the user without the need for extensive sample processing. The presence and concentration of biogenic analytes can often indicate the progress and success or failure of the production process. Beer production is no exception to this as several metabolites have been identified as indicators of beer quality. Here we demonstrate the detection and quantitation of several biogenic amines that have been shown to affect beer flavor. Using a microfluidic capillary electrophoresis (CE)-MS platform (ZipChip, 908 Devices, Boston MA) we can rapidly (<4 min) analyze a wide range of biologically relevant metabolites with virtually no sample preparation.

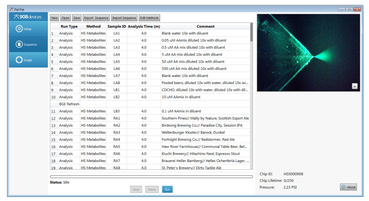
Methods

Beer samples were stored frozen until all 96 were collected, then thawed, diluted 10x in water, centrifuged to remove particulates, diluted 10x in Metabolite Sample Diluent (908 Devices, Boston, MA) containing heavy isotope labeled amino acid internal standards, and then loaded directly into a 96 well plate. The plate was then placed into an autosampler equipped microfluidic CE-ESI devices (ZipChip Interface, 908 Devices, Boston, MA) which was coupled to an orbitrap MS (Exactive Plus EMR, Thermo Scientific) for analysis with a scan range of 70-350 m/z. A microfluidic chip with a 10 cm separation channel was used (ZipChip HSX). The chip was filled with Metabolites background electrolyte (BGE) and a field strength of 1000 V/cm was applied, yielding analysis times of 4 minutes.

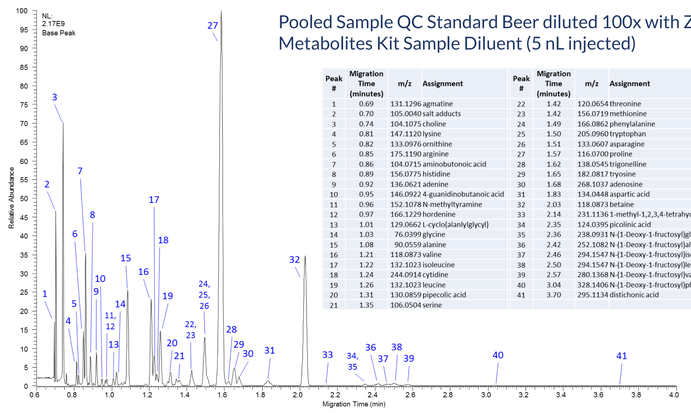
Sample preparation, analysis and data processing was all completed in less than 24 hours for the 133 ZipChip runs performed. Each analysis (5 nL injection into the separation channel) consumed 50 pL of beer. The remaining beer samples (~375 mL x 96) were disposed of in accordance with local (NC-ABC) laws via eager human waste-processing bioreactors.







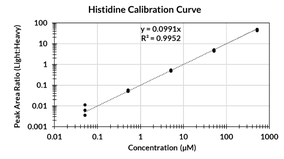
Spent Growth Media Analysis

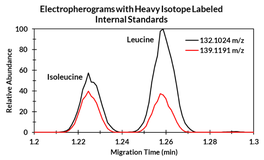


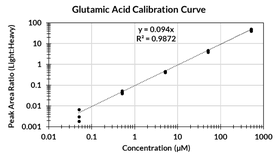
Pooled Sample QC Standard Beer diluted 100x with ZipChip Metabolites Kit Sample Diluent (5 nL injected)

Peak #	Migration Time (minutes)	m/z	Assignment	Peak #	Migration Time (minutes)	m/z	Assignment
1	0.69	131.1296	agmatine	22	1.42	120.0654	threonine
2	0.70	105.0040	salt adducts	23	1.42	156.0719	methionine
3	0.74	104.1075	choline	24	1.49	166.0862	phenylalanine
4	0.81	147.1120	lysine	25	1.50	205.0960	tryptophan
5	0.82	133.0976	ornithine	26	1.51	133.0607	asparagine
6	0.85	175.1190	arginine	27	1.57	116.0700	proline
7	0.86	104.0715	aminobutanoic acid	28	1.62	138.0545	trigonelline
8	0.89	156.0775	histidine	29	1.65	182.0817	tyrosine
9	0.92	136.0621	adenine	30	1.68	268.1037	adenosine
10	0.95	146.0922	4-guanidinobutanoic acid	31	1.83	134.0448	aspartic acid
11	0.96	152.1078	N-methyltyramine	32	2.03	118.0873	betaine
12	0.97	166.1229	hordenine	33	2.14	231.1136	1-methyl-1,2,3,4-tetrahydro-beta-carboline-3-carboxylic acid
13	1.01	129.0662	L-cyclo(alanylglycyl)	34	2.35	124.0395	picolinic acid
14	1.03	76.0399	glycine	35	2.36	238.0931	N-(1-Deoxy-1-fructosyl)glycine
15	1.08	90.0559	alanine	36	2.42	252.1082	N-(1-Deoxy-1-fructosyl)alanine
16	1.21	118.0873	valine	37	2.46	294.1547	N-(1-Deoxy-1-fructosyl)isoleucine
17	1.22	132.1023	isoleucine	38	2.50	294.1547	N-(1-Deoxy-1-fructosyl)leucine
18	1.24	244.0914	cytidine	39	2.57	280.1368	N-(1-Deoxy-1-fructosyl)valine
19	1.26	132.1023	leucine	40	3.04	328.1406	N-(1-Deoxy-1-fructosyl)phenylalanine
20	1.31	130.0859	pipecolic acid	41	3.70	295.1134	distichonic acid
21	1.35	106.0504	serine				

Amino Acid Quantitation



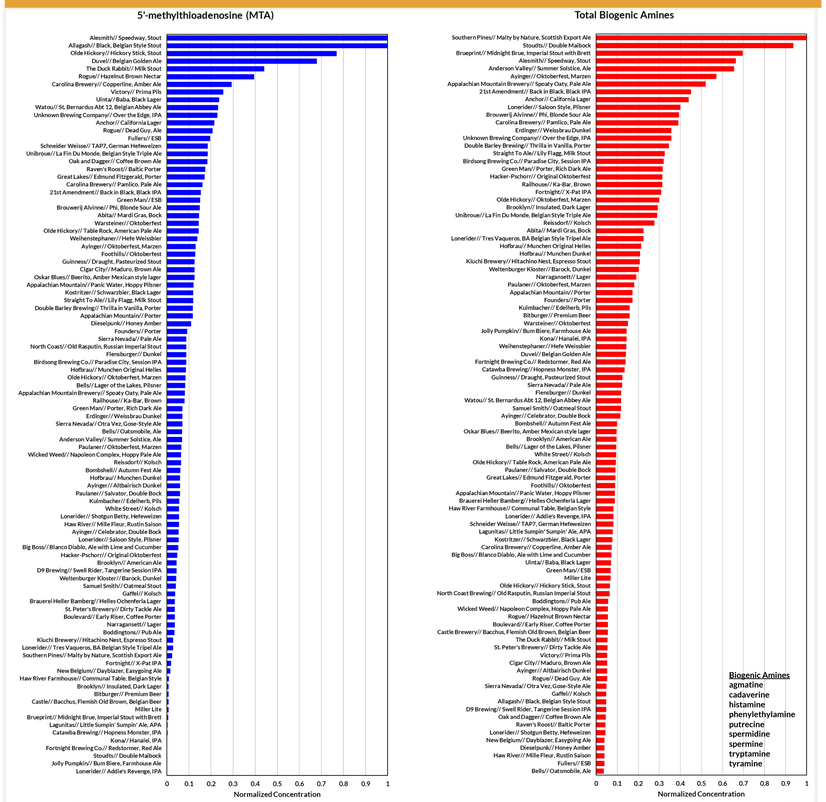




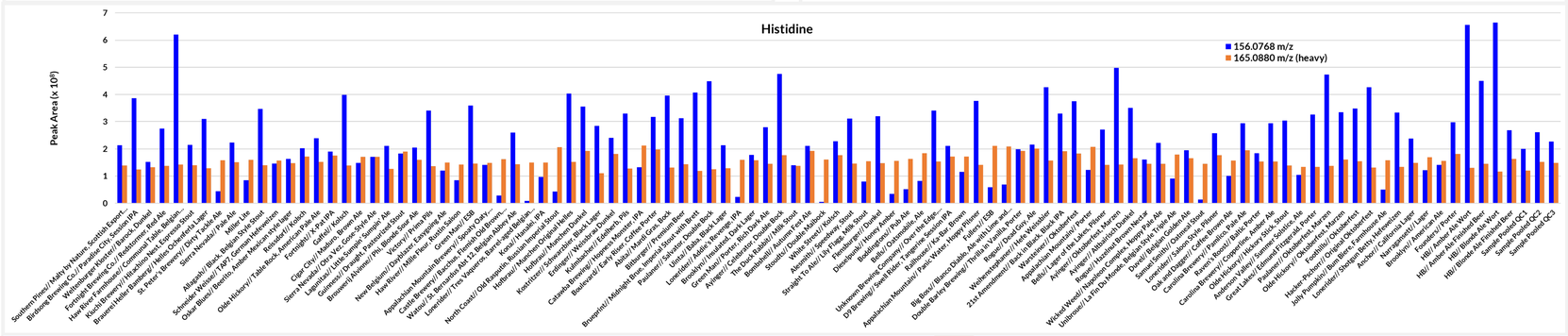
Analyte	Slope	R²
Lysine	0.0916	0.9998
Arginine	0.0987	0.9944
Histidine	0.0991	0.9952
Glycine	0.0701	0.9700
Alanine	0.0826	0.9990
Valine	0.0983	0.9914
Isoleucine	0.1146	0.9978
Leucine	0.1137	0.9991
Serine	0.0912	0.9941
Threonine	0.1030	0.9950
Methionine	0.0920	0.9957
Phenylalanine	0.0866	0.9980
Proline	0.0955	0.9963
Glutamic Acid	0.0940	0.9872
Tyrosine	0.0930	0.9992
Aspartic Acid	0.0893	0.9999

Heavy isotope labeled internal standards of the above 16 amino acids were spiked into each sample at a concentration of 1 µM. Calibration curves were plotted for each amino acid's light-to-heavy isotope peak area ratio. Histidine and Glutamic Acid calibration curves are shown at left and are representative of others produced in this experiment. Below the raw histidine peak areas are shown for each of the beer and homebrew samples for both the heavy and light isotopes.

Metabolites and Taste



MTA and biogenic amines, products of several metabolic processes of common brewing yeast and bacterial strains as well as strains that are responsible for beer spoilage, have been shown to contribute off- flavors to beers. All beers analyzed, regardless of MTA or BA levels, were found to be delicious.



Bioreactor Monitoring : Homebrew Analysis

Homebrew wort was fermented w/ *Saccharomyces cerevisiae* at ~ 5 x 10^9 cells/liter wort for 3 weeks at 17°C in a stainless steel conical fermentation vessel.

Conclusions and References

The ZipChip microfluidic CE-MS system provides an easy-to-use platform for rapid bioreactor monitoring. These analyses require little, if any, sample preparation and yield high quality MS data with hundreds of quantifiable features in a 4 minute run.

- Heuberger et al., *Food Chemistry* 200 (2016) 301–307.
- Dandrifosse et al., *Food Chemistry* 89 (2005) 519–525.
- www.908devices.com

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