

Untargeted Beer Analysis using LC-QTOF-MS – Differentiation of Beer Styles based on Phenolic and iso-alpha-Acids

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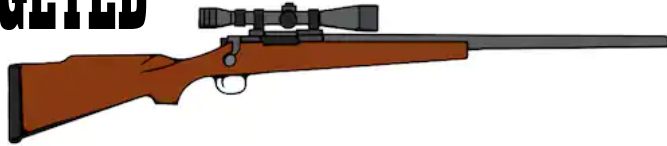
Today's Talk

- Targeted vs. Untargeted Analysis
- Analysis of Beer
- Targeting Phenolic and Iso- α -acids
- Untargeted Analysis of Beer
- Extension to Altbier



Targeted vs. Untargeted Analysis

TARGETED

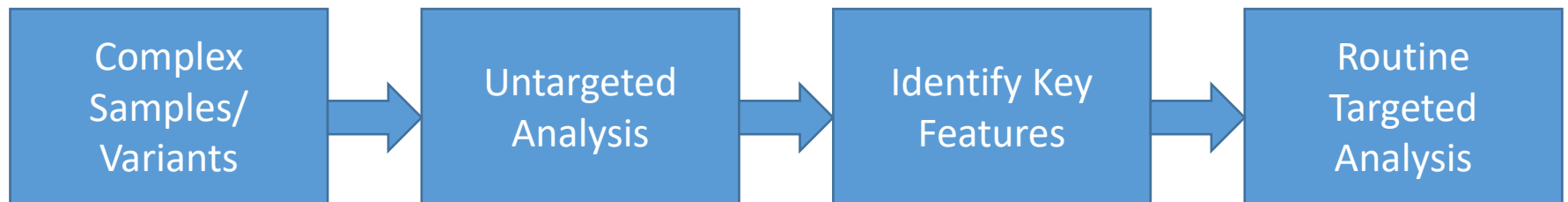


- Targets identified
- Absolute quantification
- Hypothesis driven
- High sensitivity/high specificity (MRM)
- Validation with reference standards
- Reducible to routine

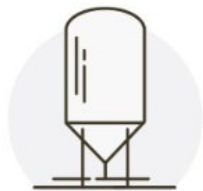
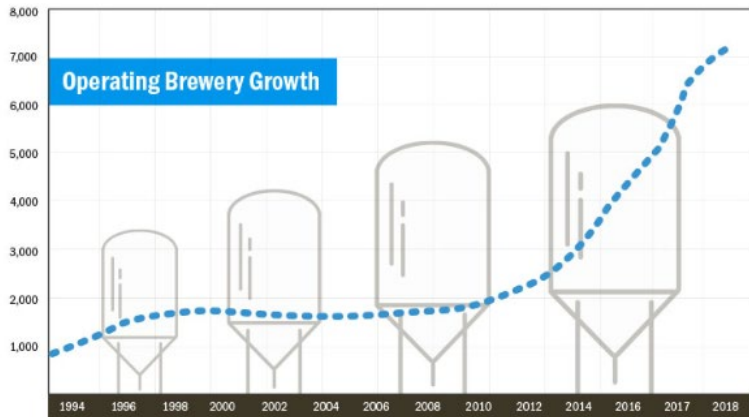


UNTARGETED

- Key features unknown
- Global/comprehensive analysis
- Hypothesis generating
- Qualitative identification
- Databases and libraries
- Complex, data rich, statistical tools



Beer

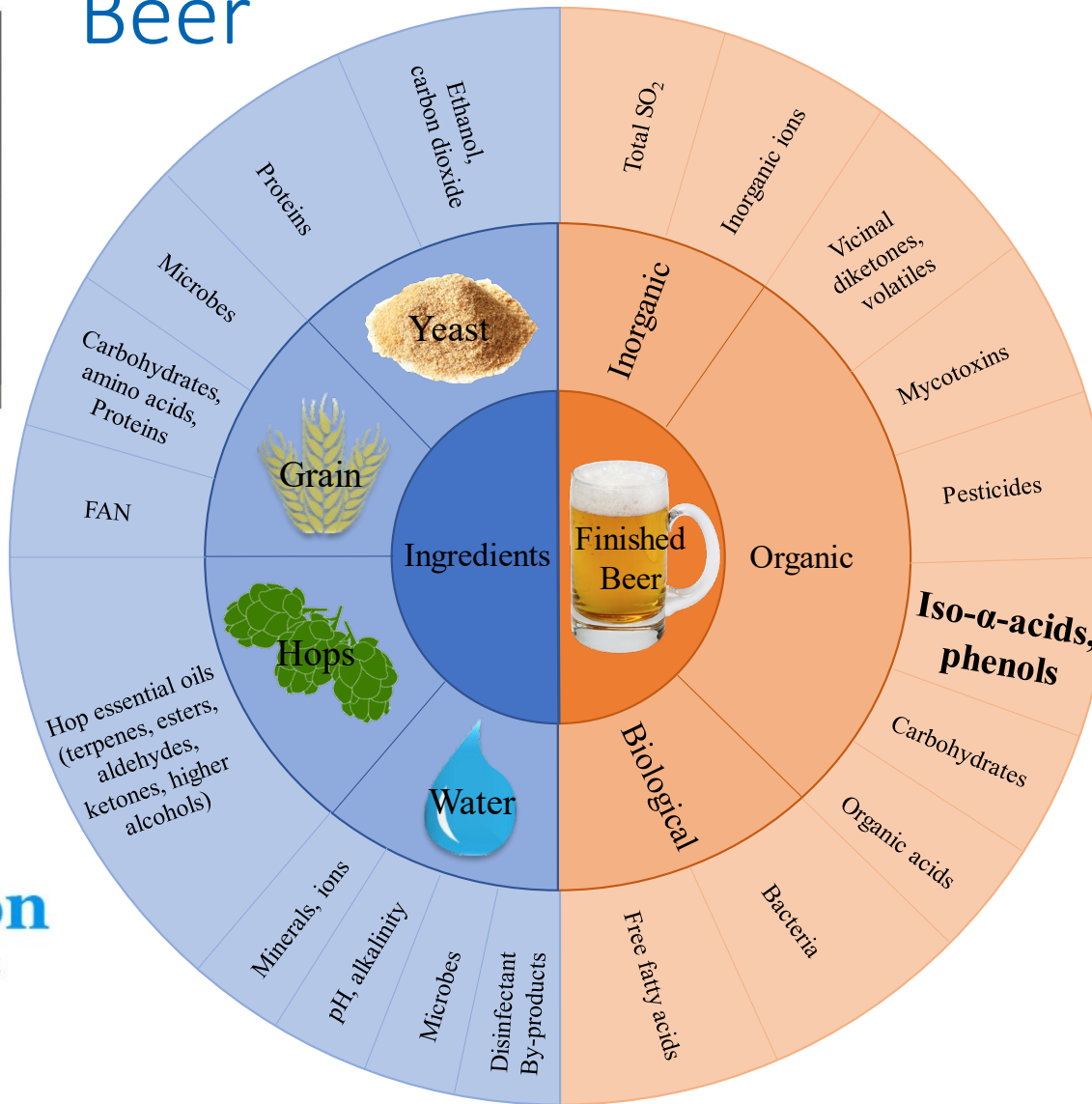


7,346
2018 U.S. Operating
Breweries



\$27.6 Billion

2018 Craft Retail Dollar Value



Anderson et al., *Anal. Chim. Acta* **2019**, 1085, 1-20.







PABST BLUE RIBBON
1844-PACK

12-OZ

\$849.99

Established in Milwaukee 1844

1844
PACK

Pabst
Blue Ribbon
BEER

Pabst
Blue Ribbon
BEER

Pabst
Blue Ribbon
BEER

Pabst
Blue Ribbon
BEER

Pabst
Blue Ribbon
BEER

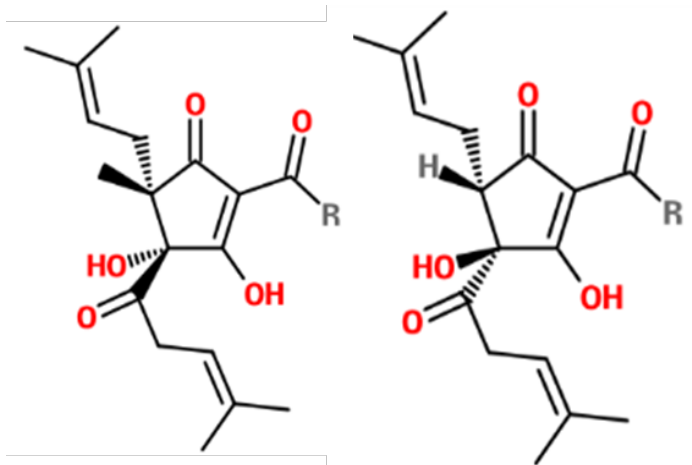
Pabst
Blue Ribbon
BEER

Pabst
Blue Ribbon
BEER

Pabst
Blue Ribbon
BEER

Pabst
Blue Ribbon
BEER

Iso- α -Acids and Phenolic Compounds



trans-

Isohumulone (IAA)

$R_1 = \text{CH}_2\text{CH}(\text{CH}_3)_2$, Isohumulone

$R_2 = \text{CH}(\text{CH}_3)_2$, isohumulone

$R_3 = \text{CH}(\text{CH}_3)\text{CH}_2\text{CH}_2$, adisohumulone

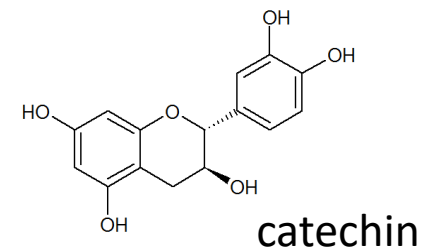
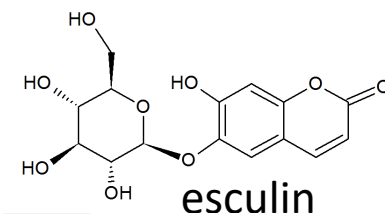
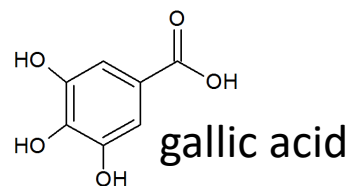
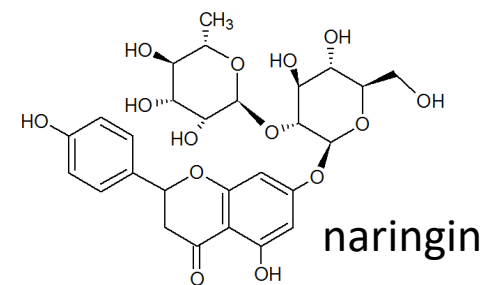
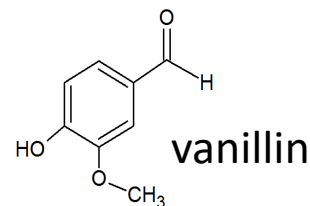
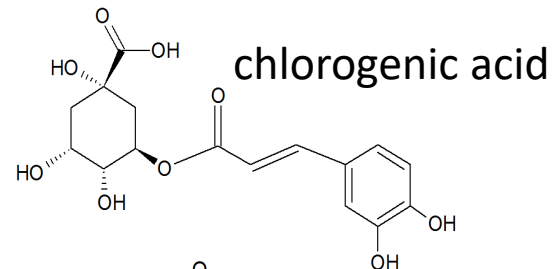
$R_4 = \text{CH}_2\text{CH}(\text{CH}_3)_2$, preisohumulone

$R_5 = \text{CH}_3$, postisohumulone

$R_6 = (\text{CH}_2)_4\text{CH}_3$, pre-postadisohumulone



cis-



Analysis

Shimadzu LCMS-9030 (QTOF)

- Raptor C18 (12 min)
- RP gradient (Water/CAN, 1% FA)
- Negative ionization mode ESI



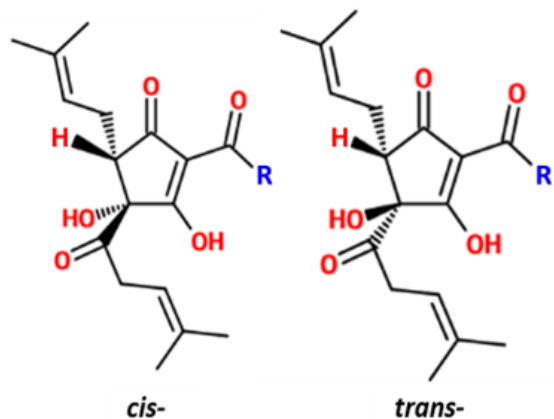
35 Beers, 5 Styles

- Sonicate 30 min, dilute 50% with water

Restek Raptor C18 (100 x 2.1, 2.7 μ), 0.4 mL/min, water/ACN + 1% FA



Iso- α -Acids

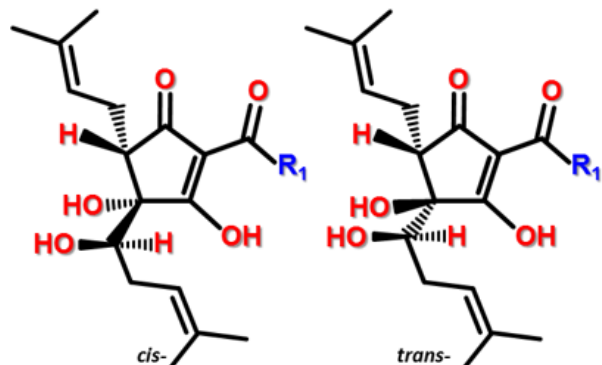


Isohumulones (IAA)

$R_1 = \text{CH}_2\text{CH}(\text{CH}_3)_2$, Isohumulone

$R_2 = \text{CH}(\text{CH}_3)_2$, Isocohumulone

$R_3 = \text{CH}(\text{CH}_3)\text{CH}_2\text{CH}_3$, Isoadhumulone



(S)-Rho-isohumulones (R1iAA)

[R1iH] Rho-1-isohumulone

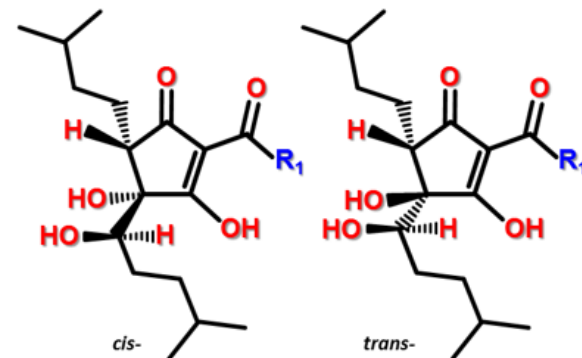
[R1iCH] Rho-1-isocohumulone

[R1iAH] Rho-1-isoadhumulone

$R_1 = \text{CH}_2\text{CH}(\text{CH}_3)_2$

$R_1 = \text{CH}(\text{CH}_3)_2$

$R_1 = \text{CH}(\text{CH}_3)\text{CH}_2\text{CH}_3$



(S)-Hexahydro-isohumulones (H1iAA)

[H1iH] Hexahydro-1-isohumulone

[H1iCH] Hexahydro-1-isocohumulone

[H1iAH] Hexahydro-1-isoadhumulone

$R_1 = \text{CH}_2\text{CH}(\text{CH}_3)_2$

$R_1 = \text{CH}(\text{CH}_3)_2$

$R_1 = \text{CH}(\text{CH}_3)\text{CH}_2\text{CH}_3$

[R2iH] Rho-2-isohumulone

[R2iCH] Rho-2-isocohumulone

[R2iAH] Rho-2-isoadhumulone

$R_1 = \text{CH}_2\text{CH}(\text{CH}_3)_2$

$R_1 = \text{CH}(\text{CH}_3)_2$

$R_1 = \text{CH}(\text{CH}_3)\text{CH}_2\text{CH}_3$

[H2iH] Hexahydro-2-isohumulone

[H2iCH] Hexahydro-2-isocohumulone

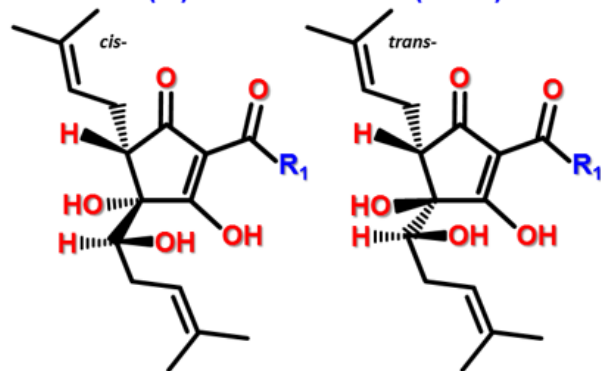
[H2iAH] Hexahydro-2-isoadhumulone

$R_1 = \text{CH}_2\text{CH}(\text{CH}_3)_2$

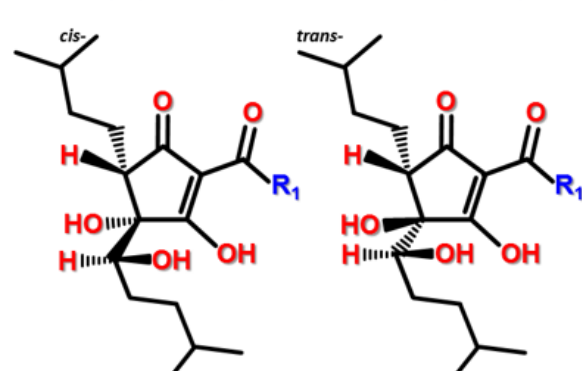
$R_1 = \text{CH}(\text{CH}_3)_2$

$R_1 = \text{CH}(\text{CH}_3)\text{CH}_2\text{CH}_3$

(R)-Rho-isohumulones (R2iAA)



(R)-Hexahydro-isohumulones (H2iAA)



Tetrahydro-isohumulones (TiAA)

[TiH] Tetrahydro-isohumulone

[TiCH] Tetrahydro-isocohumulone

[TiAH] Tetrahydro-isoadhumulone

$R_1 = \text{CH}_2\text{CH}(\text{CH}_3)_2$

$R_1 = \text{CH}(\text{CH}_3)_2$

$R_1 = \text{CH}(\text{CH}_3)\text{CH}_2\text{CH}_3$



Iso- α -Acids High-Res MRMs

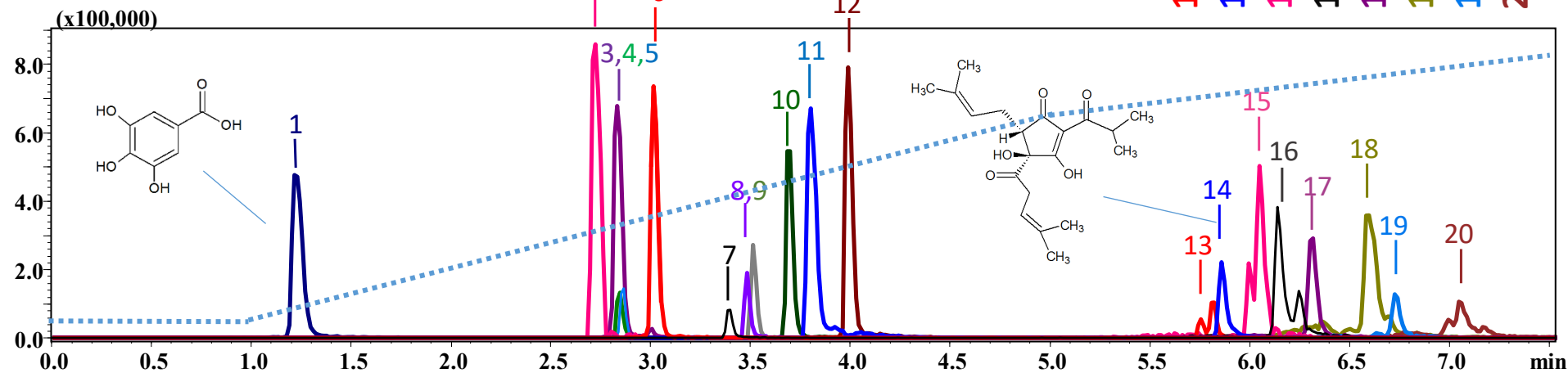
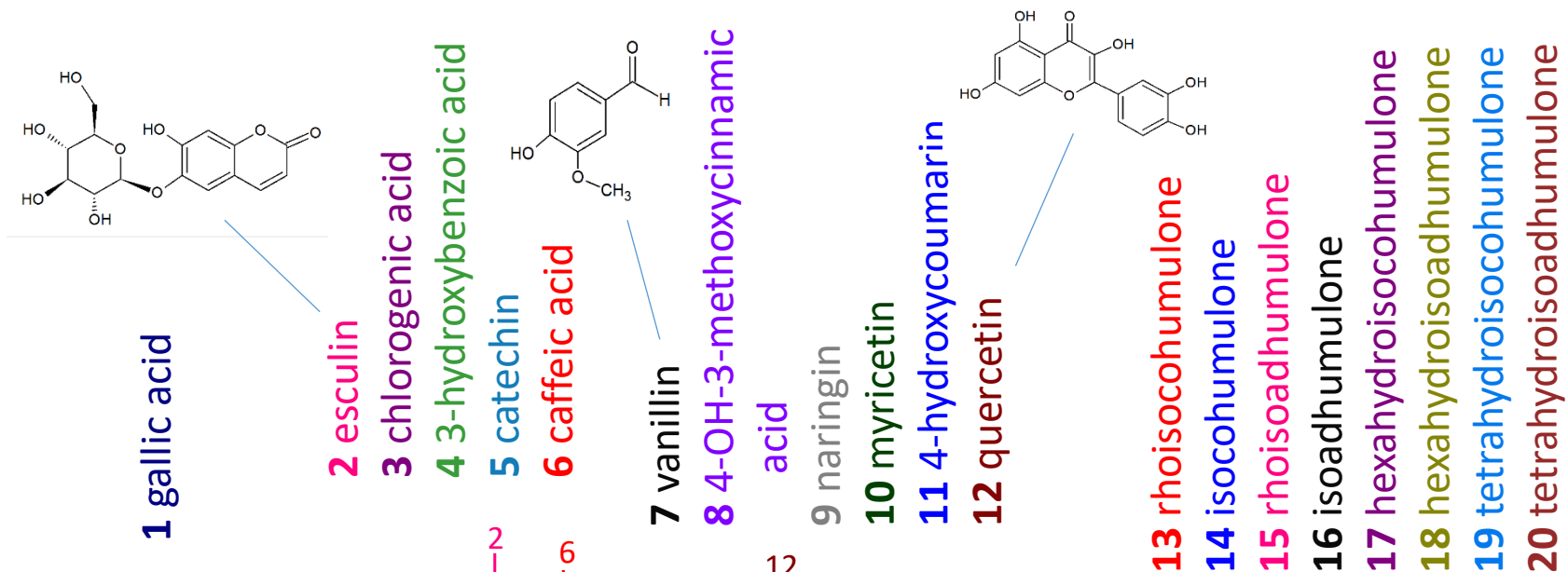
Analyte	Group	ID	Standard Retention Time (min)	Parent Mass (m/z)	Product Ions (m/z)	CE [V]
trans-isohumulone	IAA	t-iH	6.089	361.2011	96.0758, 265.1474	15.0
cis-isohumulone		c-iH		361.2011	96.0758, 265.1474	15.0
trans-isocohumulone		t-iCH	5.675	347.1852	182.0597, 251.1311	21.0
cis-isocohumulone		c-iCH		347.1852	182.0597, 251.1311	21.0
trans-isoadhumulone		t-IAH	5.968	361.2011	96.0758, 265.1474	15.0
cis-isoadhumulone		c-IAH		361.2011	96.0758, 265.1474	15.0
trans-rho-isohumulone	RiAA	t-RiH		363.2179	196.0766, 247.1367	22.0
cis-rho-isohumulone		c-RiH		363.2179	196.0766, 247.1367	22.0
trans-rho-isocohumulone		t-RiCH		349.2015	182.0598, 233.1202	21.0
cis-rho-isocohumulone		c-RiCH	5.755, 5.821	349.2015	182.0598, 233.1202	21.0
trans-rho-isoadhumulone		t-RiAH		363.2179	196.0766, 247.1367	22.0
cis-rho-isoadhumulone		c-RiAH	5.995, 6.061	363.2179	196.0766, 247.1367	22.0
trans-tetra-isohumulone	TiAA	t-TiH	7.185	365.2324	249.1523, 267.0000	25.0
cis-tetra-isohumulone		c-TiH		365.2324	249.1523, 267.0000	25.0
trans-tetra-isocohumulone		t-TiCH	6.450	351.2165	239.1316, 253.0000	22.0
cis-tetra-isocohumulone		c-TiCH	6.542	351.2165	239.1316, 253.0000	22.0
trans-tetra-isoadhumulone		t-TiAH	7.000	365.2324	249.1523, 267.0000	25.0
cis-tetra-isoadhumulone		c-TiAH	7.050	365.2324	249.1523, 267.0000	25.0
trans-hexa-isohumulone	HiAA	t-HiH		367.2483	223.1729, 249.1526	25.0
cis-hexa-isohumulone		c-HiH	6.700	367.2483	223.1729, 249.1526	25.0
trans-hexa-isocohumulone		t-HiCH		353.2324	235.1333, 253.0000	22.0
cis-hexa-isocohumulone		c-HiCH	6.321	353.2324	235.1333, 253.0000	22.0
trans-hexa-isoadhumulone		t-HiAH		367.2483	223.1729, 249.1526	25.0
cis-hexa-isoadhumulone		c-HiAH	6.500, 6.600	367.2483	223.1729, 249.1526	25.0

Phenolics High-Res MRMs

Analyte	Standard Retention Time (min)	Parent Mass (m/z)	Product Ions (m/z)	CE [V]
Catechin	2.647	289.0708	123.0441, 203.0702	22.0
Caffeic Acid	2.801	179.0344	134.0383, 135.0465	21.0
Chlorogenic Acid	2.598	353.0928	161.0252, 191.0559	22.0
Cinnamic Acid	3.310	193.0497	134.0382, 178.0288	18.0
Benzoic Acid	2.592	137.0239	65.0387, 93.0345	25.0
Quercetin	3.882	301.0336	151.0014, 178.9998	21.0
Naringin	3.367	579.1631	151.0008, 271.0563	28.0
Myricetin	3.564	317.0288	137.0248, 151.0043	19.0
Vanillin	3.187	151.0395	108.0223, 136.0175	19.0
Hydroxycoumarin	3.655	161.0245	117.0341	21.0
Gallic Acid	1.069	169.0138	79.0180, 125.0222	18.0
Esculin	2.457	339.0713	133.0299, 177.0216	22.0



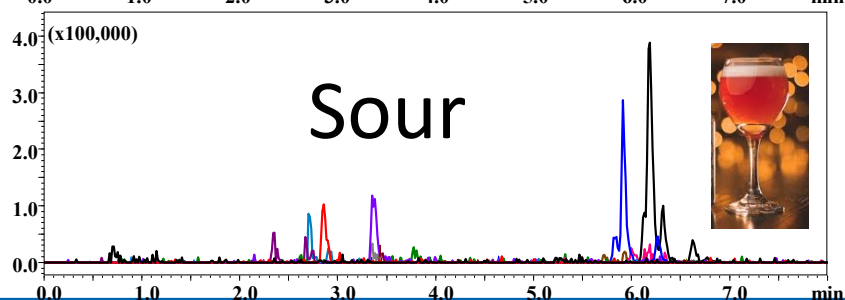
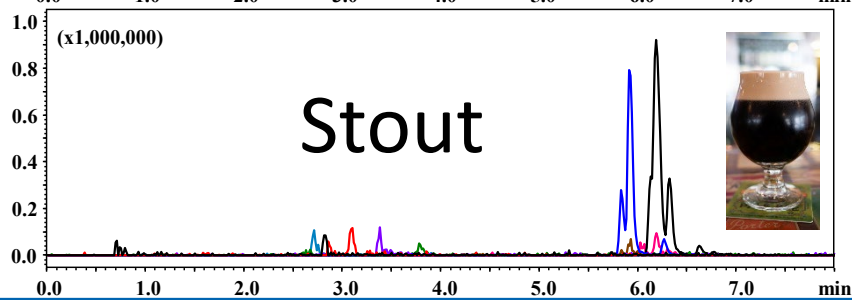
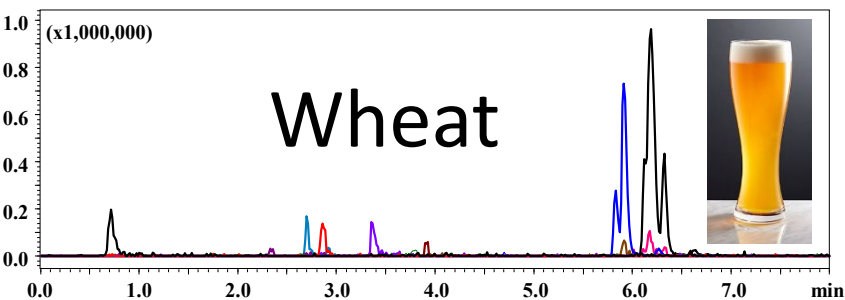
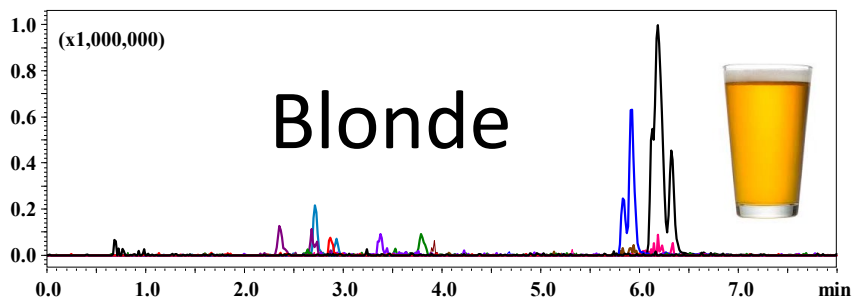
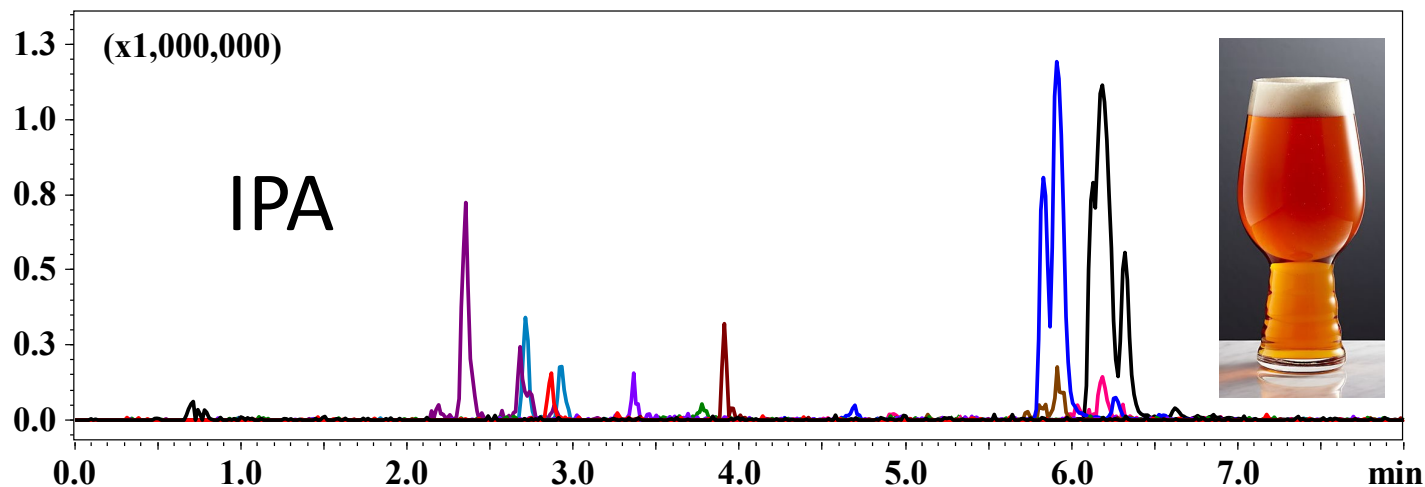
Separation of Standards



Restek Raptor C18 (100 x 2.1, 2.7 μ), 0.4 mL/min, water/ACN + 1% FA



High-Resolution MRMs

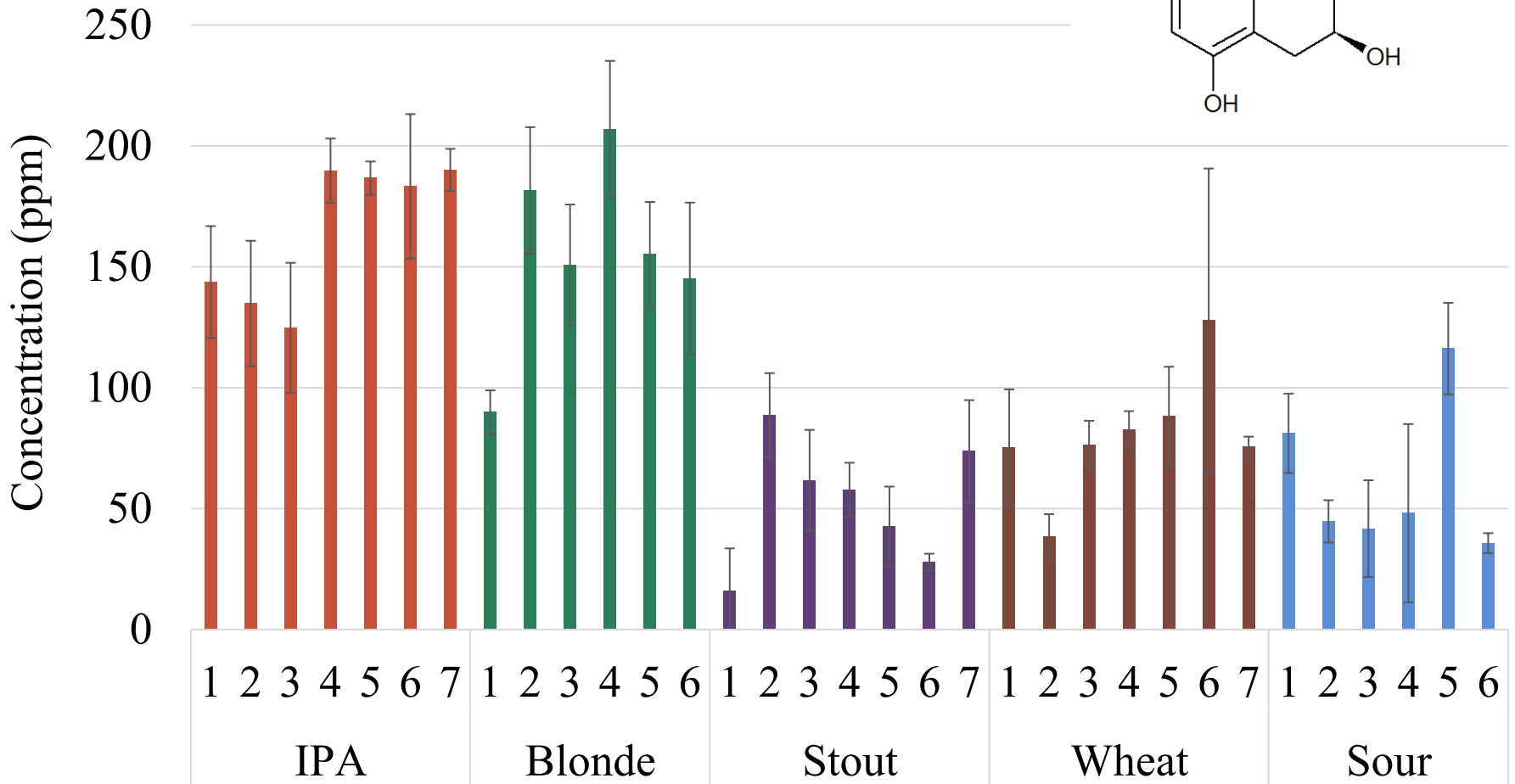
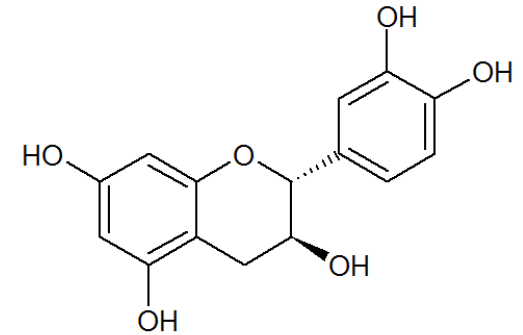




IPA
LOT

Catechin

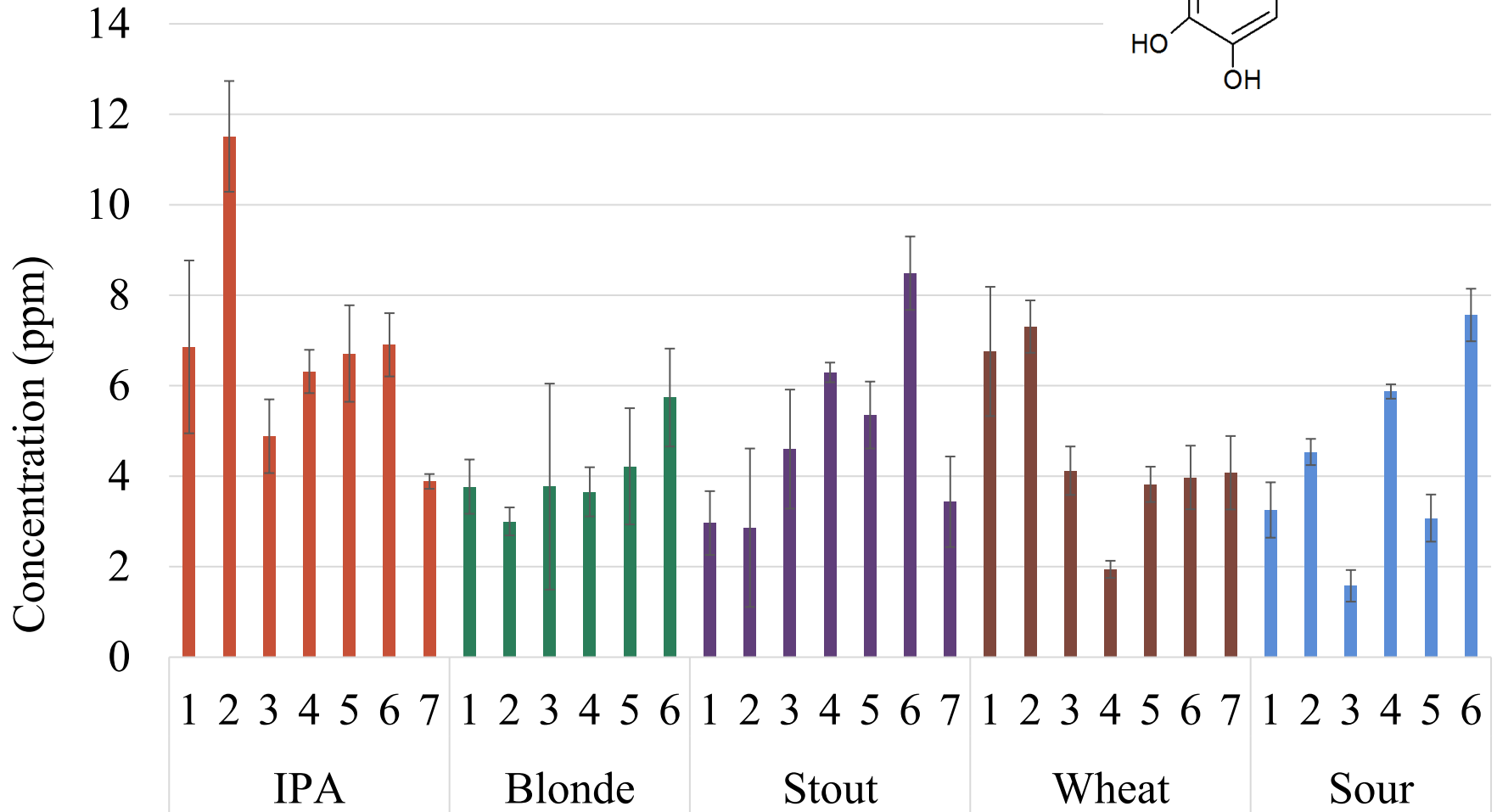
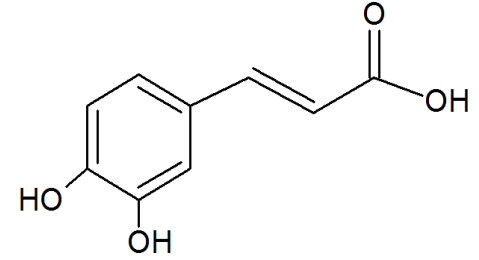
flavan-3-ol antioxidant



Green tea contains 200 – 400 ppm catechin



Caffeic Acid

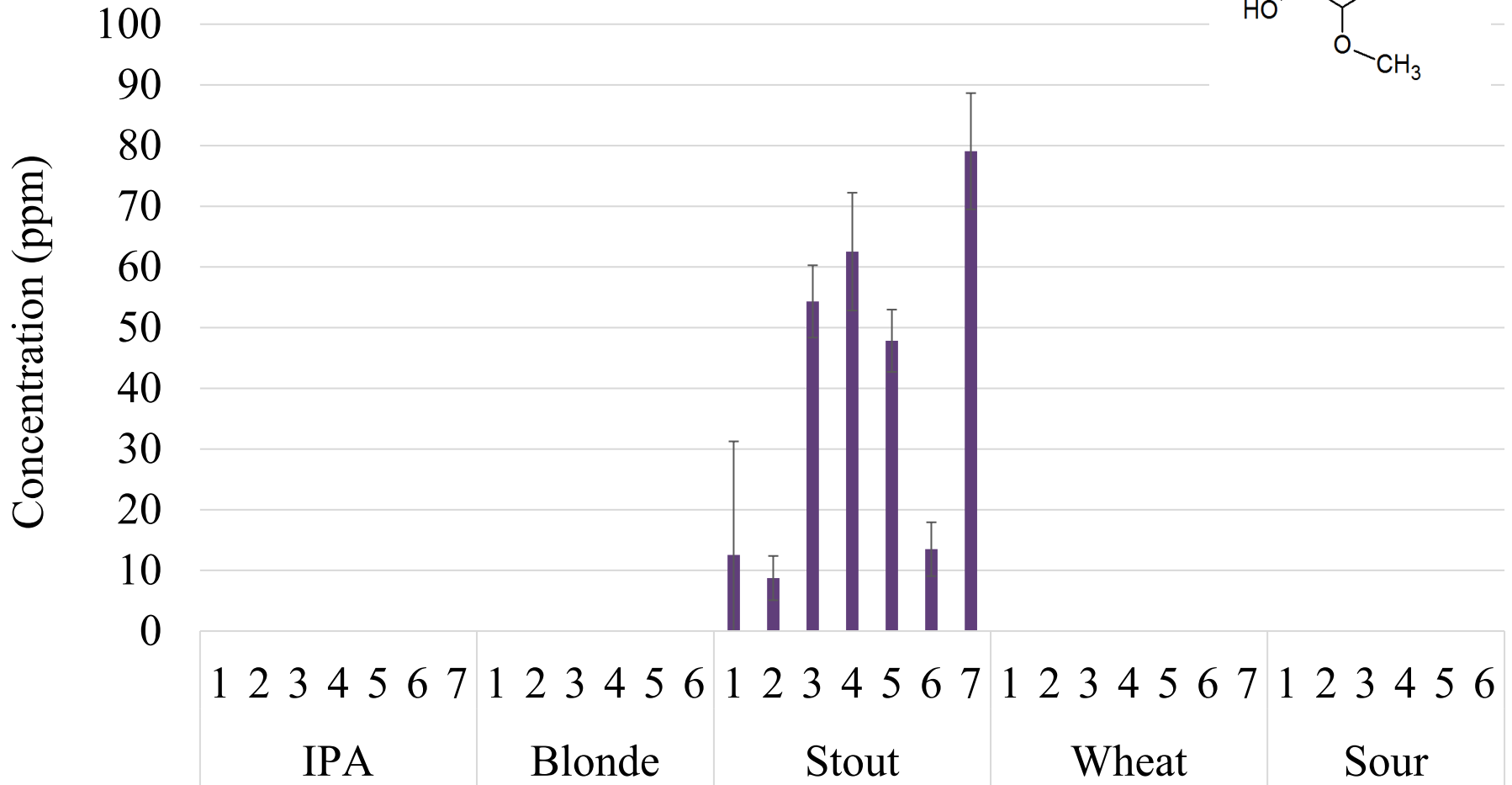
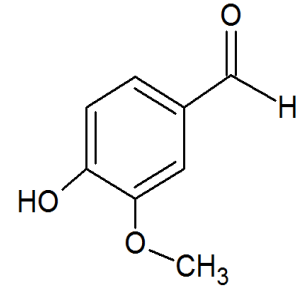


Key intermediate in the formation of higher molecular weight phenolic compounds (e.g. chlorogenic acids)



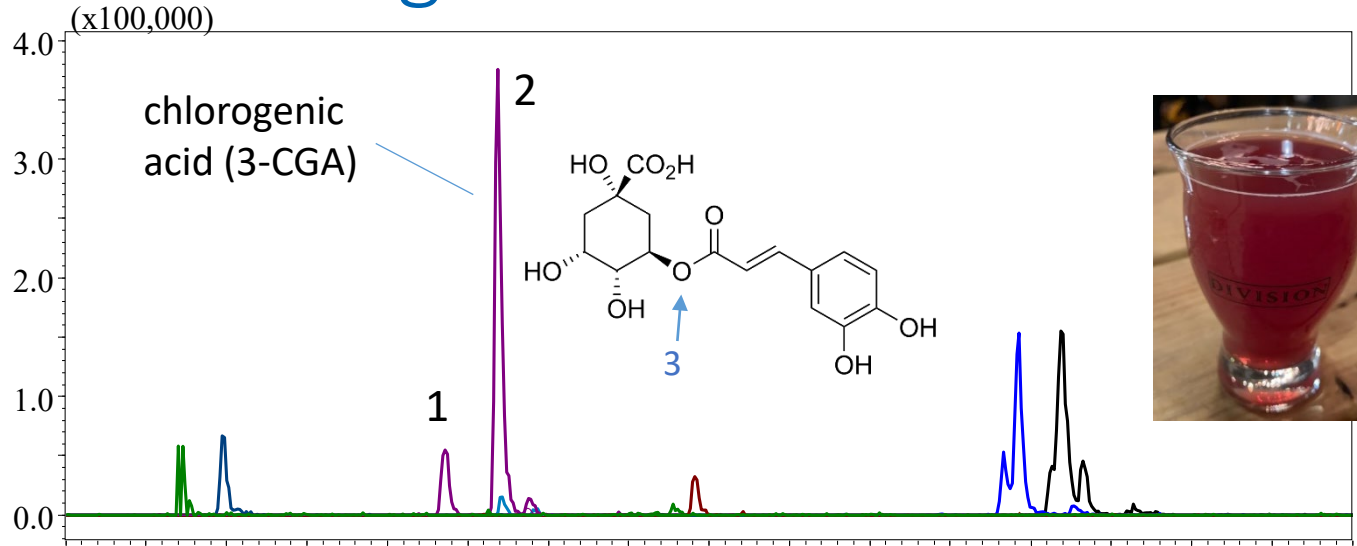
Vanillin

Flavor/Fragrance

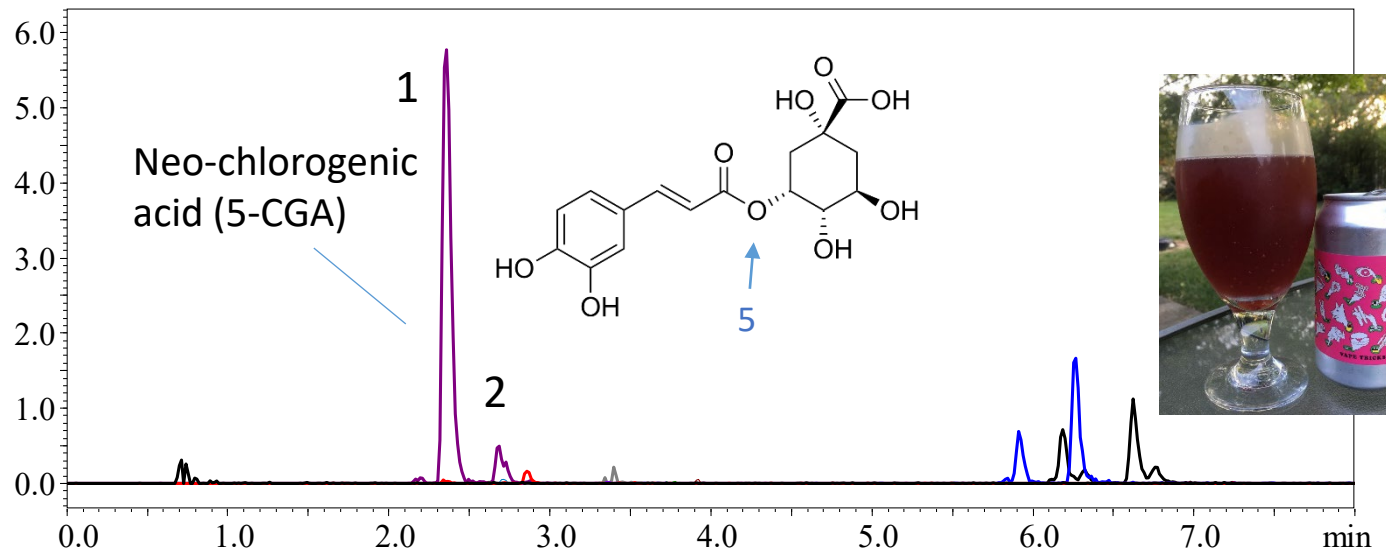


Chlorogenic Acid

“Elder Bramble”
(Division Brewing)
Sour ale made
with elder flower
and blackberries



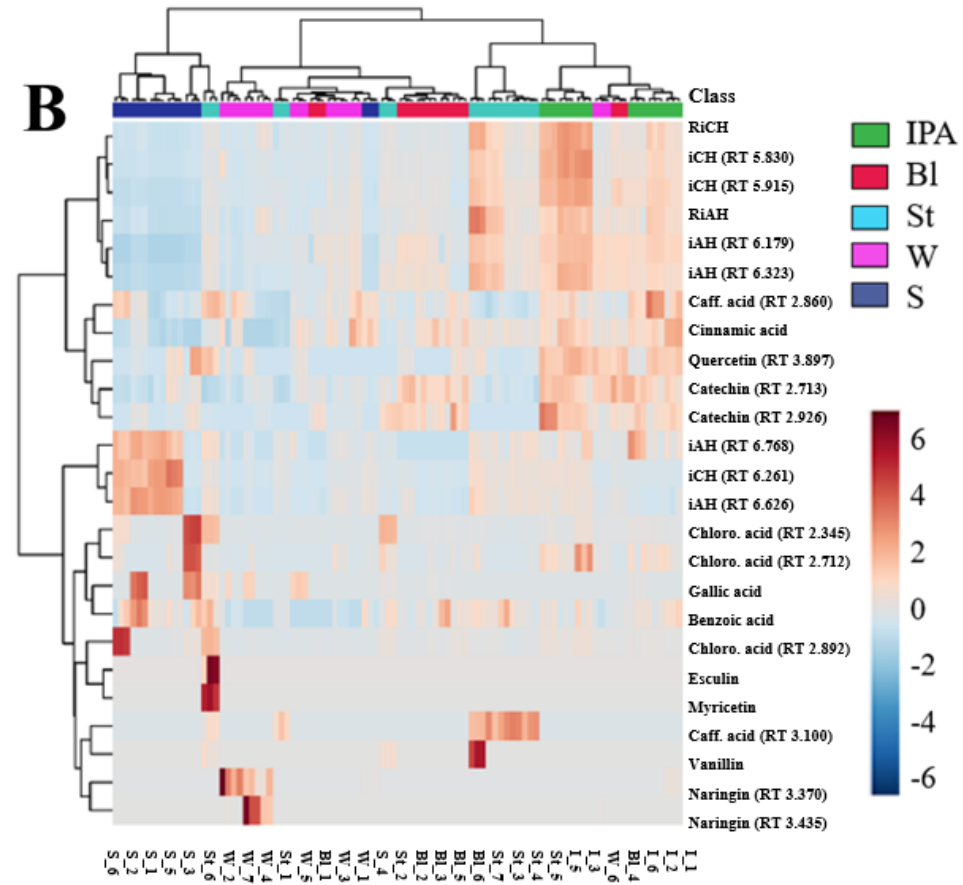
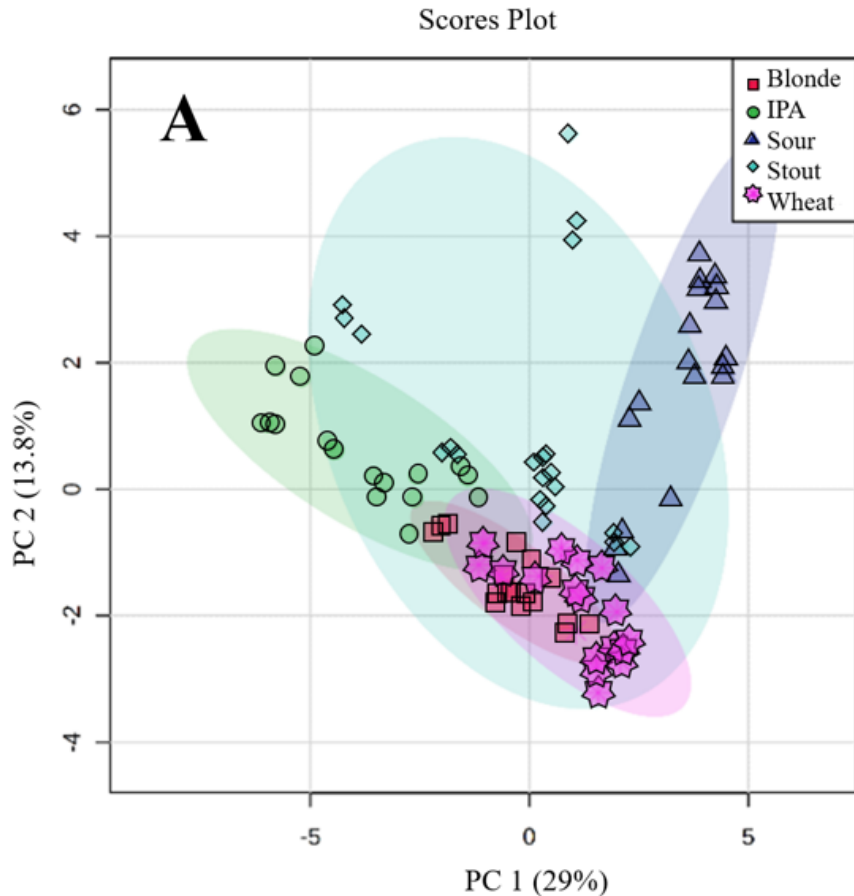
“Vape Tricks”
(Prairie Artisan Ales)
Sour ale aged on
cherries



Blackberries have higher 3-CGA than 5-CGA; cherries, the opposite
(*J. Agric. Food Chem.* **2012**, *60*, 5716-5727; Cherries:..., **2017** (CAB International))



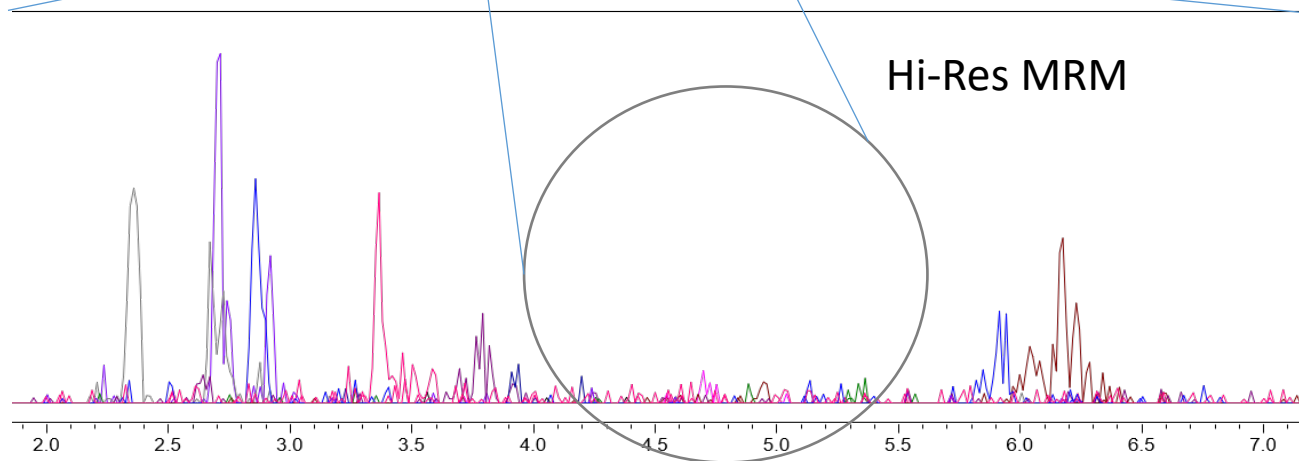
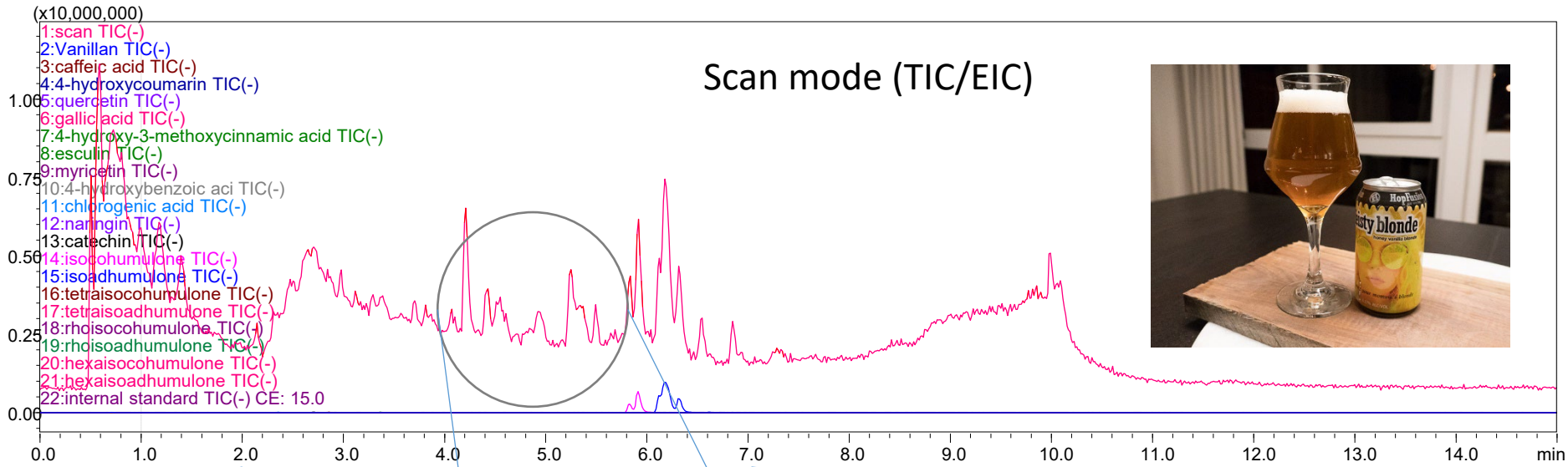
Targeted Differentiation



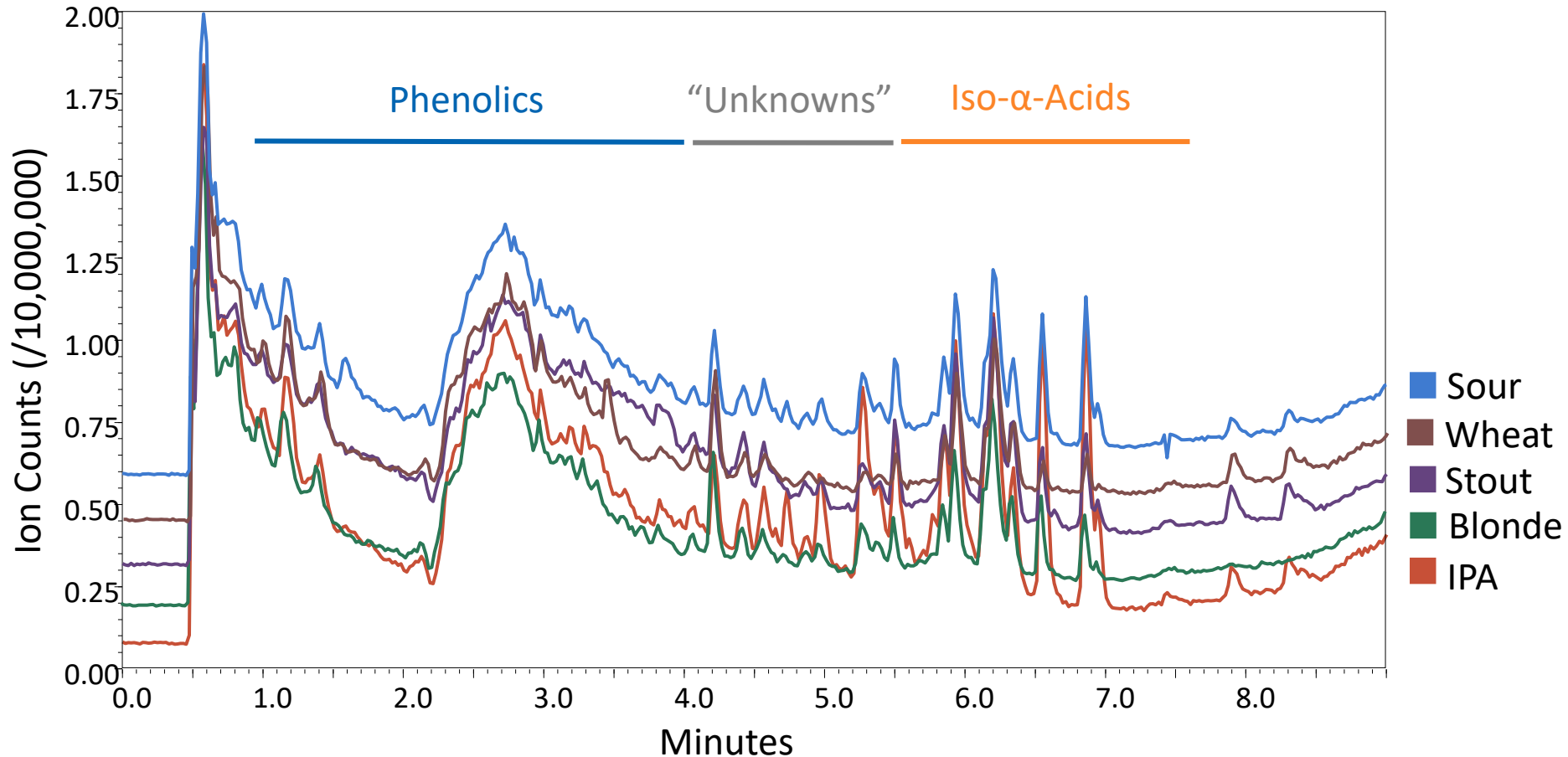
Total of 36 targeted compounds



Feisty Blonde – A Case for Untargeted Analysis



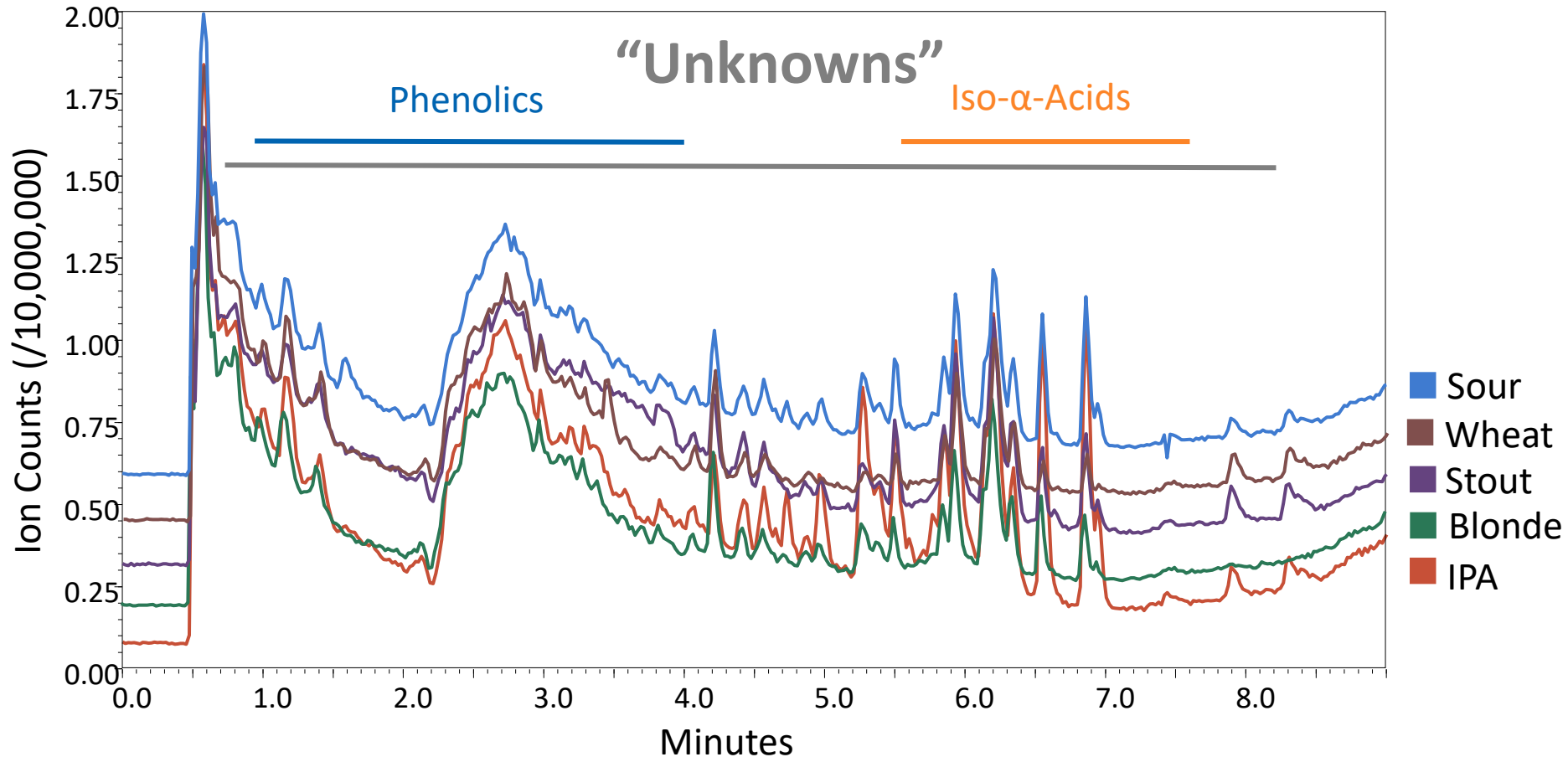
Total Ion Chromatograms



QC ("equimixture") samples for each style



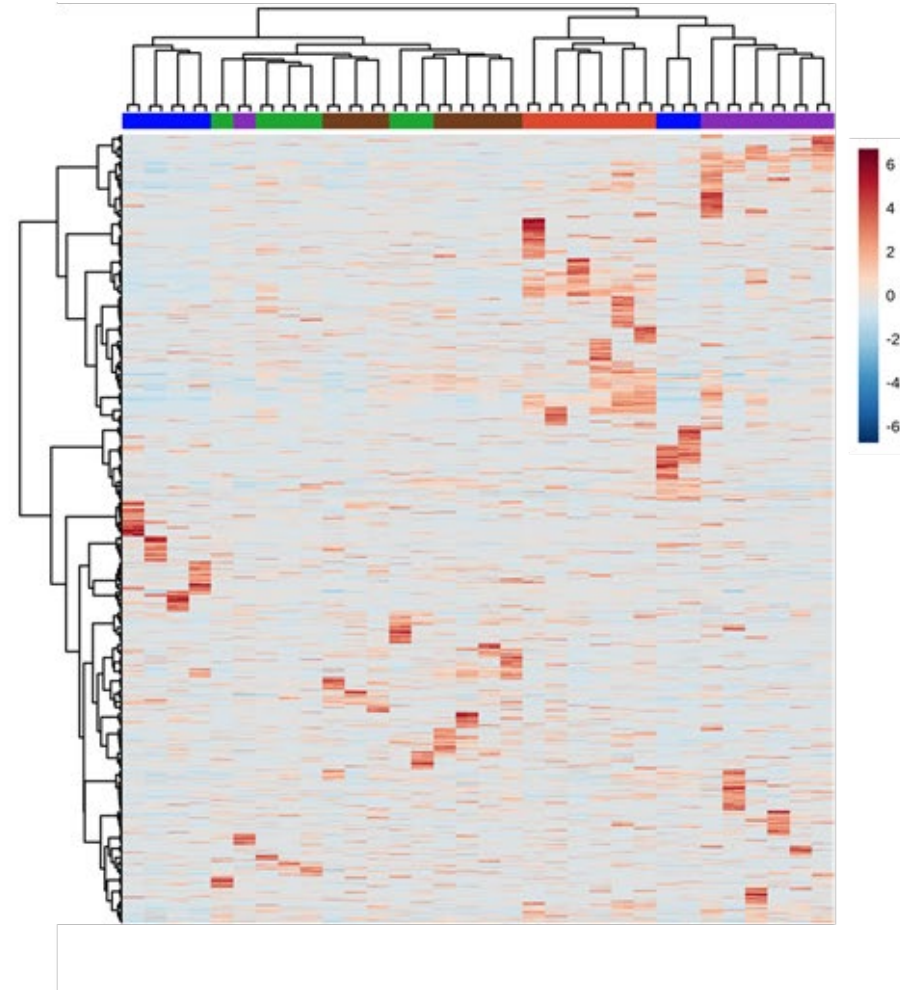
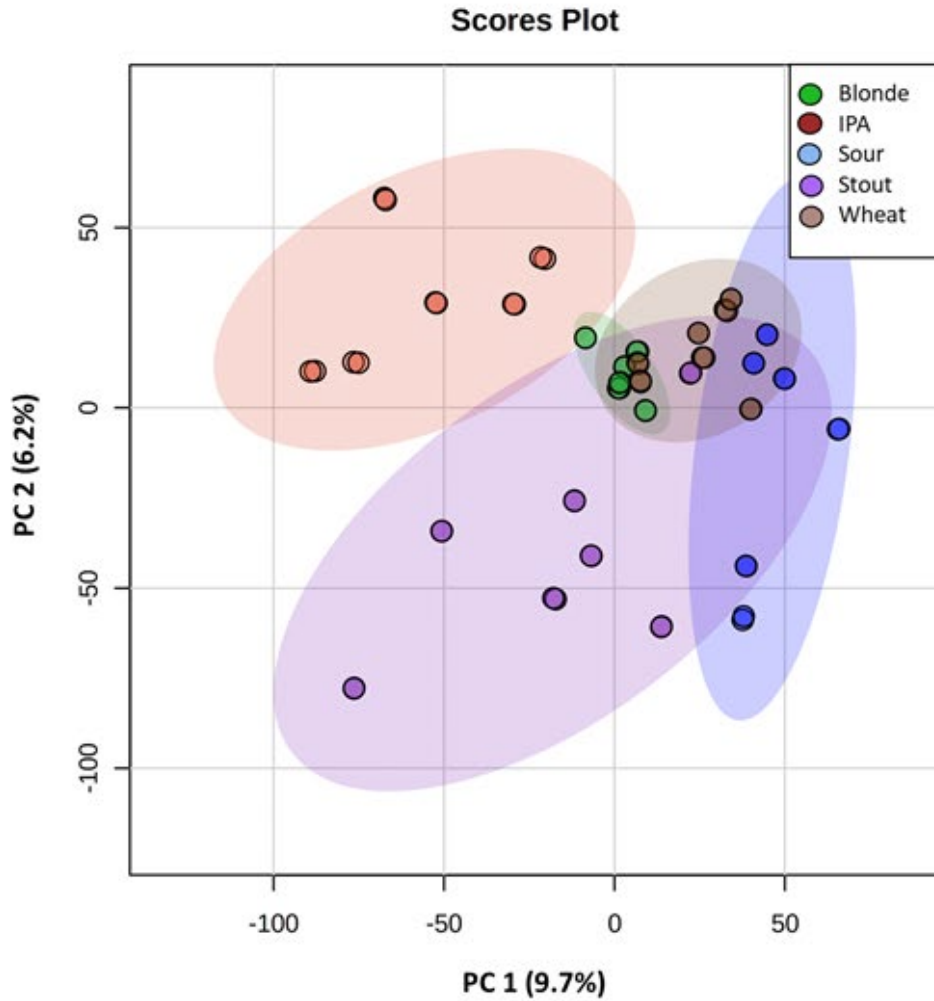
Total Ion Chromatograms



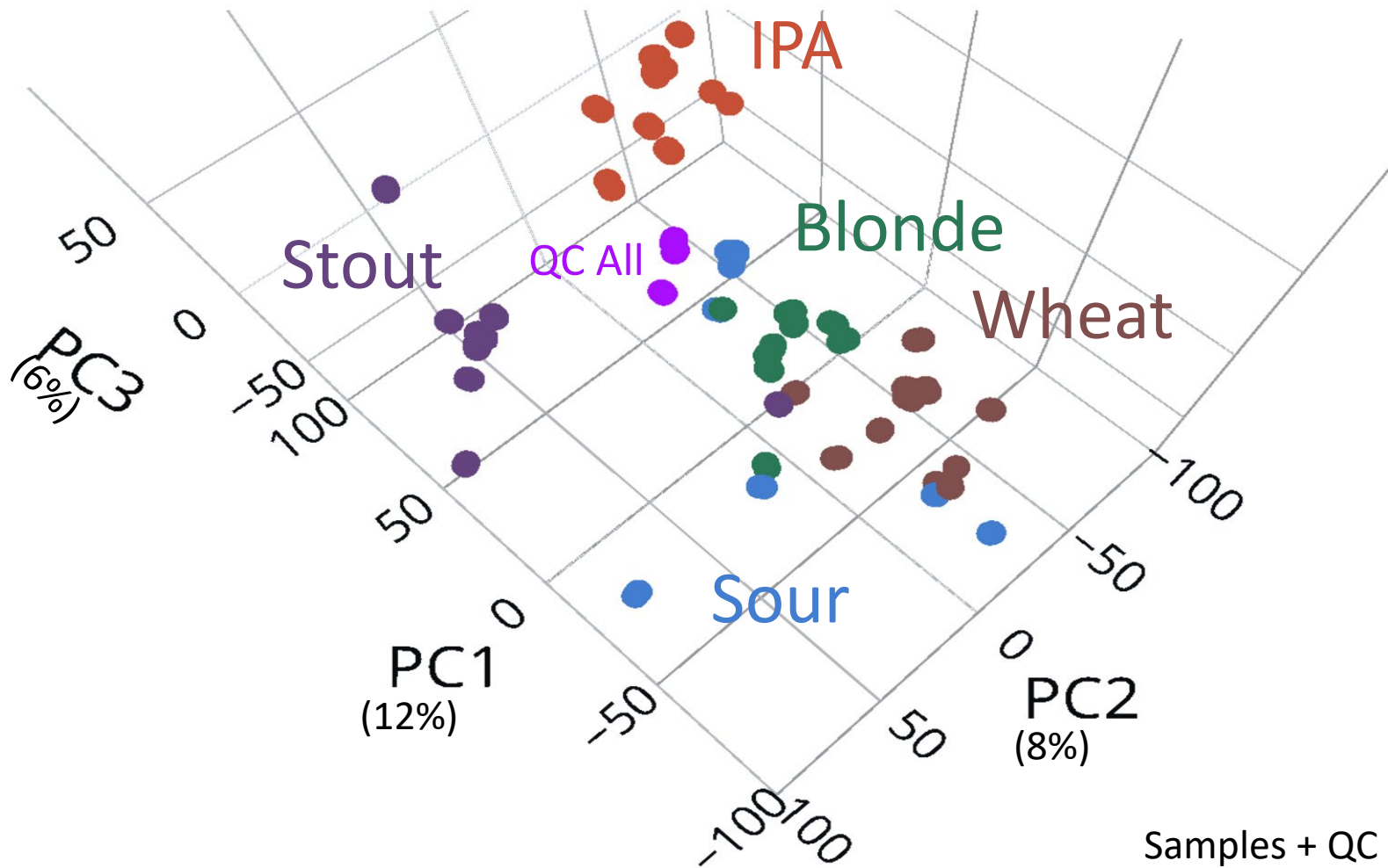
QC ("equimixture") samples for each style



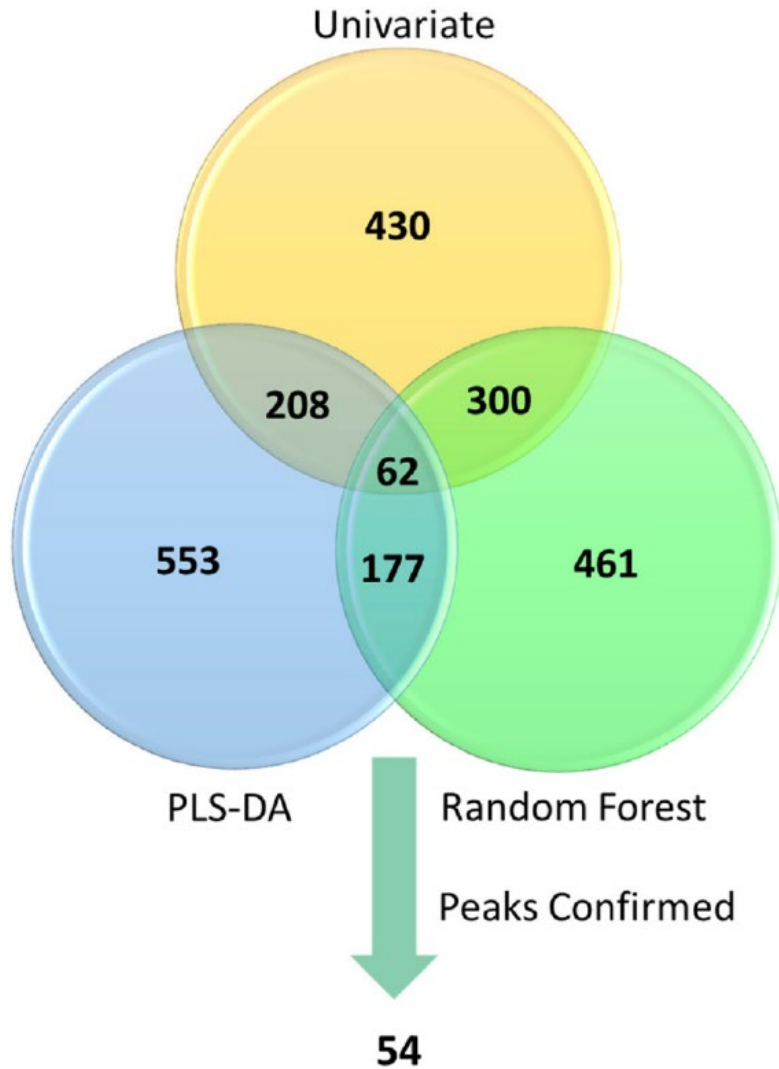
Untargeted Differentiation



Untargeted Differentiation



Feature Selection



- MS Dial → Metaboanalyst 5.0
- Three approaches:
 - Univariate (ANOVA)
 - PLS-DA
 - Random Forest
- 22,000 features → 54



Key Features; Tentative ID

Style	RT (min)	Accurate Mass	Formula	Error (mDa)	Structural ontology
Blonde, IPA	0.92	159.0317	$C_6H_8O_5$	-1.8	Medium-chain keto acids and derivatives
Sour (elevated levels)	0.83	187.0277	$C_7H_8O_6$	-2.9	Tricarboxylic acids and derivatives
IPA	5.26	193.0906	$C_{11}H_{14}O_3$	-3.5	Methoxyphenols
Sour (elevated levels)	2.29	219.0542	$C_{14}H_8N_2O$	2.2	Indolonaphthyridine alkaloids
Wheat	0.65	256.1693	$C_{17}H_{23}NO$	1.4	Styrenes
Blonde	1.25	304.1088	$C_{18}H_{15}N_3O_2$	0.4	Quinazolinamines
Blonde, IPA	5.38	317.2608	$C_{20}H_{34}N_2O$	-1.0	Aminopiperidines
Wheat	2.83	369.2702	$C_{22}H_{34}N_4O$	-4.2	Naphthalenes
IPA	8.33	387.2244	$C_{17}H_{32}N_4O_6$	0.5	Aminocyclitol glycosides
Stout, Wheat	2.74	388.0950	$C_{21}H_{15}N_3O_5$	-1.1	Diarylethers
Sour, Wheat	7.06	417.2718	$C_{24}H_{38}N_2O_4$	4.1	Diterpenoids
Wheat	2.47	450.3019	$C_{29}H_{41}NO_3$	-0.5	Steroid esters
Stout, Sour, Wheat	1.02	481.2138	$C_{30}H_{30}N_2O_4$	-0.5	Pyranoquinolines
Wheat	0.68	482.1287	$C_{21}H_{25}NO_{12}$	1.7	Flavones
Blonde, IPA	0.60	566.1679	$C_{29}H_{29}NO_{11}$	-1.1	Depsidones and depsidones



Altbier

- Originates from Rhineland, especially near Dusseldorf
- Dark copper-colored top-fermented beer
- Some fruitiness and bitterness



Experimental Design

LCMS-9030 QTOF

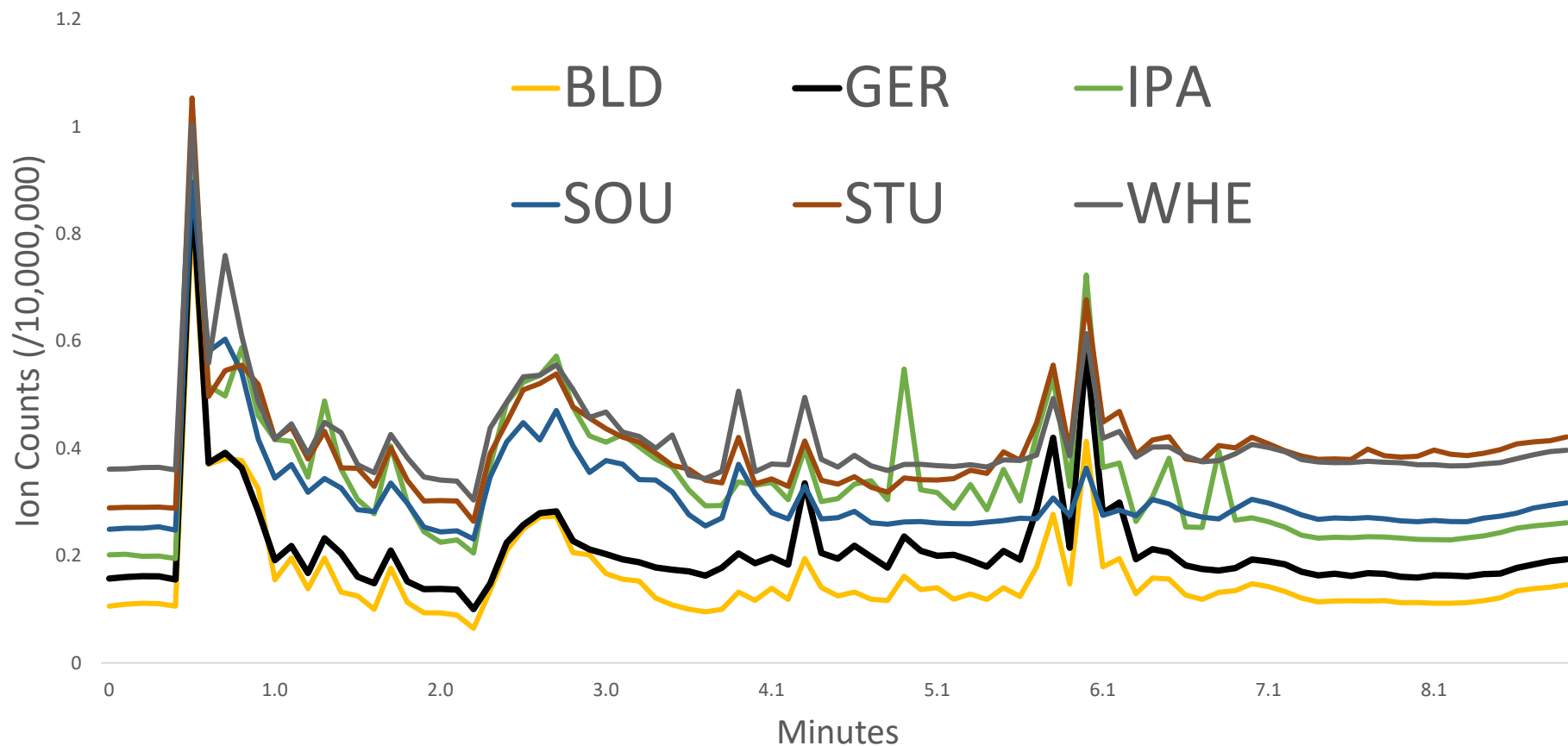
- RP gradient, Raptor ARC-C18 (12 min)
- Negative ionization mode ESI
- Open, sonicate (degas), dilute 50%
- Duplicate analysis
- QC samples: All equimix, class equimixes

Beers:

- 6 Altbier
- 2 Blonde
- 2 IPA
- 2 Stout
- 2 Wheat
- 2 Sour

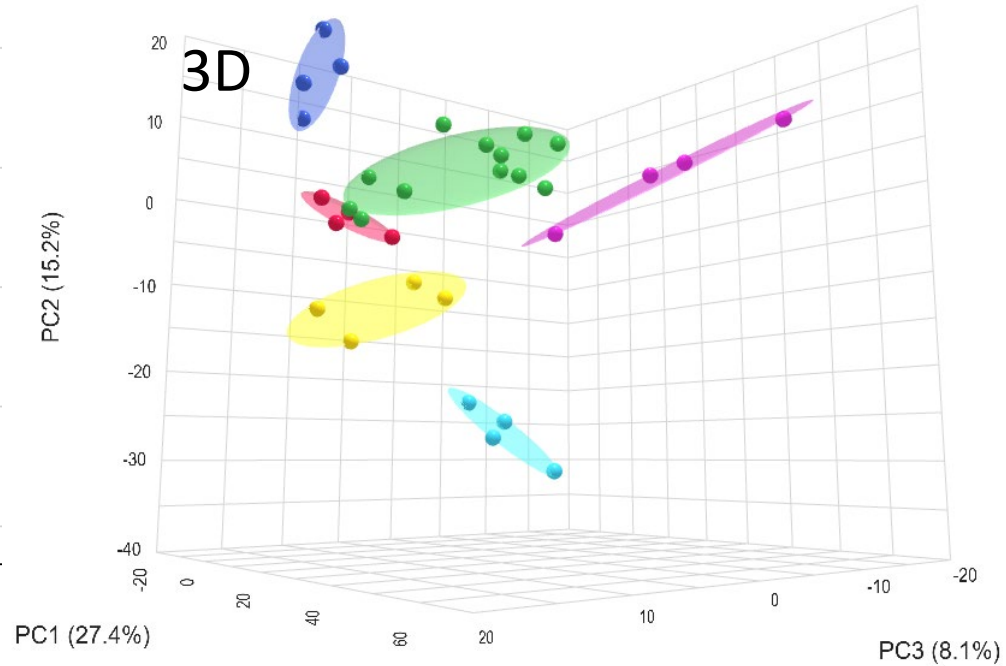
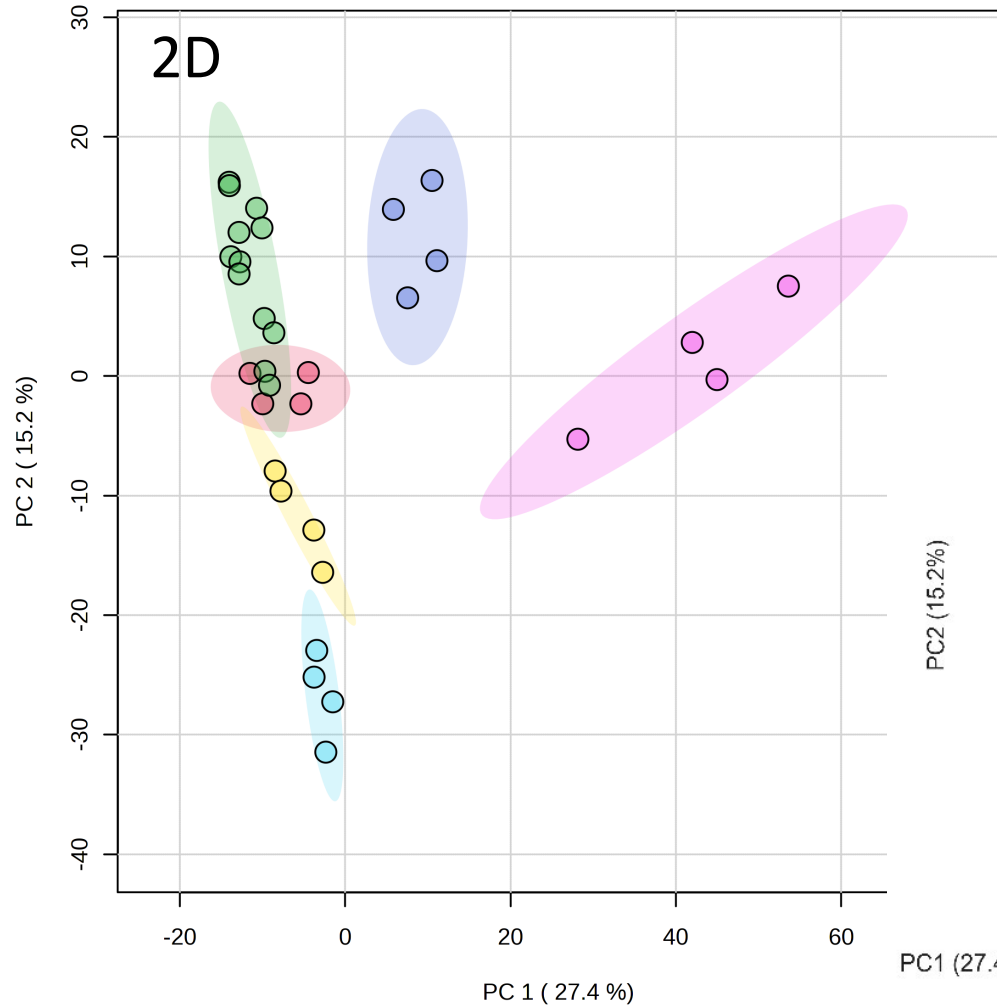


Total Ion Current



Principal Component Analysis

Scores Plot



Altbier Features

m/z	formula	Error (ppm)	name	ontology
361.2092	C ₂₁ H ₃₀ O ₅	-19.7822	Isohumulone A	Monocyclic monoterpenoids
376.1771	C ₂₀ H ₂₇ N ₀ O ₆	-1.5292	unknown	Guaianes
390.1908	C ₂₁ H ₂₉ N ₀ O ₆	3.6917	unknown	N-acyl-alpha amino acids
390.1909	C ₂₁ H ₂₉ N ₀ O ₆	3.3865	unknown	N-acyl-alpha amino acids
391.1958	C ₁₇ H ₃₂ N ₂ O ₆ S	-12.6416	MINEs-279240	Thioglycosides
428.0397	C ₂₅ H ₃₆ O ₅	1.403	Manoalide	Diterpene lactones
458.1769	C ₂₀ H ₂₅ N ₇ O ₆	5.4139	5-Methyltetrahydrofolic acid	Tetrahydrofolic Acids
458.178	C ₂₀ H ₂₅ N ₇ O ₆	3.023	5-Methyltetrahydrofolic acid	Tetrahydrofolic Acids
498.3416	C ₂₉ H ₄₅ N ₃ O ₄	-15.6648	cyclobutanecarboxamide	Macrolactams
945.47	C ₄₈ H ₆₆ N ₈ O ₁₂	2.8892	Lyngbyastatin 7	Cyclic depsipeptides

Feature selection using PLS-DA



LC-QTOF-MS of Beer

- Beer is rich in different sample dimensions – phenolics and acids are an important one
- Targeted and untargeted evaluation of craft beer phenolics and acids – benefit from data richness of untargeted analysis
- But, challenge! Identification of key unknowns that serve as classification features – need HRMS and database help
- For the “craft” beer revolution – High resolution analysis allows molecular fingerprinting for product innovation and protection, as well as quality control



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