Monitoring of the Brewing Process with GC-MS

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INTRODUCTION

Many chemical changes occur during the brewing process as raw materials are taken through various stages to yield a finished product. Understanding these changes and when they happen has the potential to direct process optimization, improve the final product, and improve efficiency of the process. Many of the changes throughout the brewing process can be detected in the aroma profile, which is comprised of the volatile and semi-volatile analytes associated with each sample. Gas chromatography coupled with mass spectrometry (GC-MS) is well-suited for analysis of volatile and semi-volatile analytes and is a powerful way to screen the aroma profile. We use GC-MS in this work to probe five points throughout the brewing process. Samples were collected pre-boil, post-boil, from a full fermenter, at the end of fermentation, and from a full bright beer tank. In this work, GC-MS was paired with headspace solid phase micro-extraction (HS-SPME) as the sampling technique to collect and concentrate the volatile and semi-volatile analytes from the headspace prior to injection. Individual analytes that were collected on the SPME fiber subsequently separate from each other as they travel through GC column. MS detection then provides information for identification and relative quantitation. Hundreds of analytes were detected in these samples and information for representative analytes is presented here. A variety of compound types including esters, terpenes, terpenoids, organic acids, alcohols, aldehydes, ketones, furans, aromatics, nitrogen-containing, and sulfur-containing analytes are presented. Various trends can be observed in these analytes, many that can be connected to the brewing process. For example, some analytes from the malt are observed to decrease during the boil while analytes from the hops are observed to increase during the boil. Ethanol was observed to increase during fermentation. Some esters increase during the boil while others increase during fermentation. Observing changes in the aroma profile overall can provide good insight to the chemical changes occurring throughout the process.

METHOD

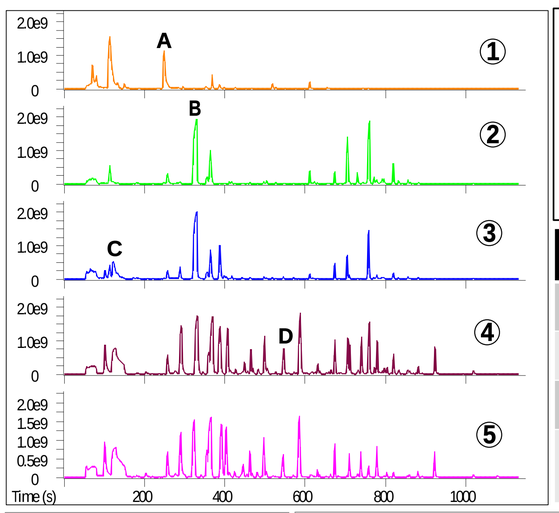
Samples were collected at various stages of the brewing process (pre-boil, post-boil, fermenter full, end of fermentation, and bright beer full tank). Each sample (5 mL in a 20 mL vial) was analyzed with HS-SPME coupled to LECO's Pegasus BT GC-TOFMS, with the method conditions listed in Table 1. An alkane standard was also acquired to calculate retention indices.

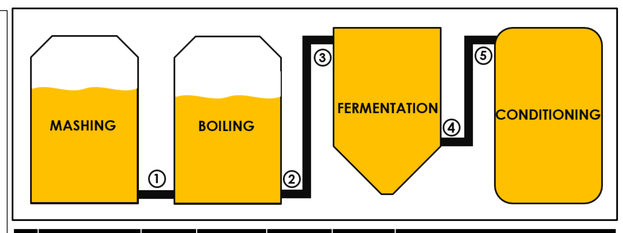
Table 1. Instrument Conditions

AS	**LECO L-PAL3 Autosampler**
SPME	10 min incubation, 20 min extraction at 35 °C
SPME fiber	DVB/Car/PDMS (Supelco)
Fiber Conditioning	5 min pre-injection at 250 °C
GC	
Injection	Desorb fiber 3 min at 250 °C, splitless
Columns	Stabilwax 30 m x 0.25 mm i.d. x 0.25 µm coating (Restek)
Carrier Gas	He @ 1.40 mL/min
Oven Program	3 min 40 °C, ramp 10 °C/min to 250 °C, hold 1 min
MS	**LECO Pegasus® BT**
Ion Source Temp	250 °C
Mass range	33-500 m/z
Acquisition Rate	10 spectra/s

GC-TOFMS Results

Representative chromatograms for each sample from the brewing processing are shown in Figure 1. Many differences are readily apparent, indicating significant chemical changes over the brewing process. Information for four analytes (labeled A-D) that clearly change is compiled in Figure 1. Other important differences may be obscured by coelutions in the total ion chromatogram (TIC) view, but are revealed with automated data processing and deconvolution, as shown In Figure 2.





	Name (CAS)	Sim	R.T. (s)	RI	Lib. RI	Notes:
A	hexanal (66-25-1)	949	248.7	1081.3	1083	aldehyde from malt decreases after boil
B	β-myrcene (123-35-3)	926	328.5	1155.6	1161	terpene extracted from hops during boil
C	ethanol (64-17-5)	937	124.8	939.3	932	ethanol formed by yeast during fermentation
D	ethyl octanoate (106-32-1)	928	585.8	1431.6	1435	ester formed by yeast during fermentation

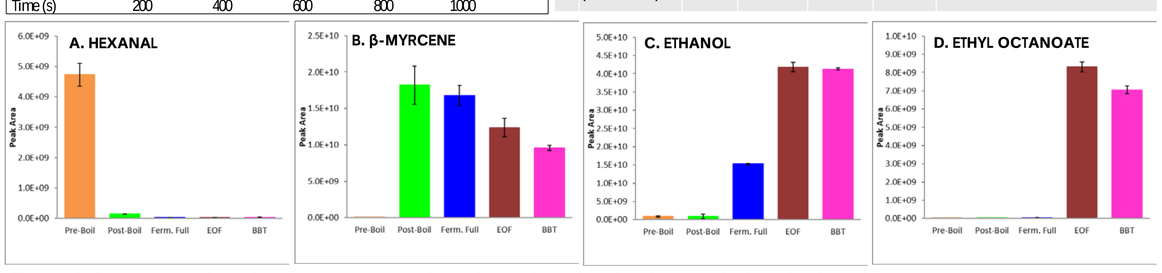


Figure 1. Representative chromatograms and analytes that clearly change during the brewing process.

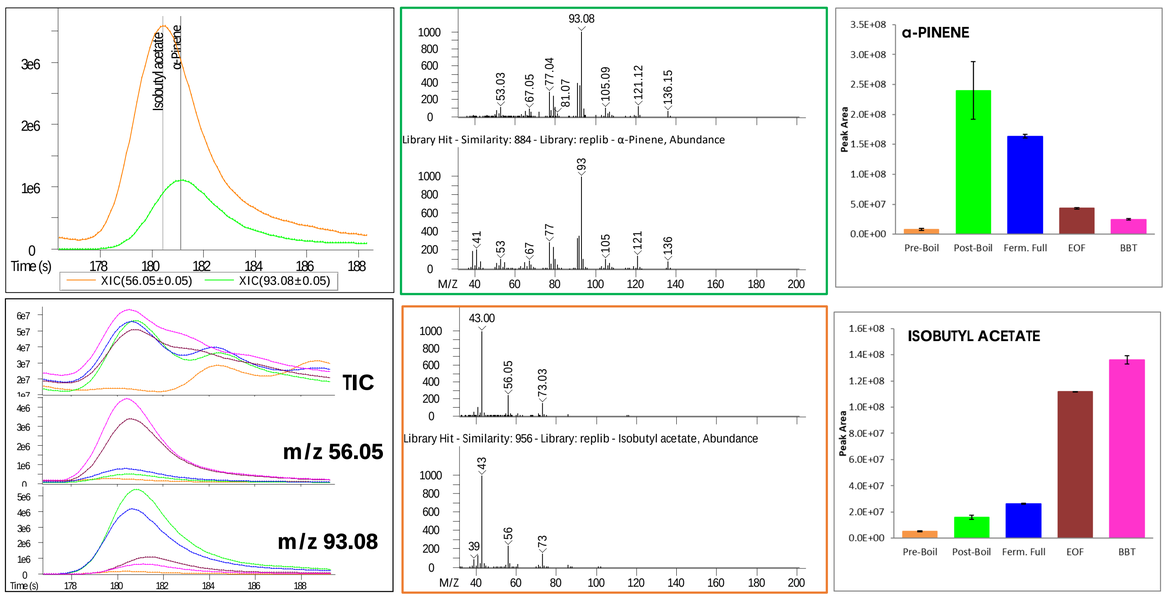


Figure 2. The trends for other analytes may be obscured by coelutions in the TIC view. In these cases, deconvolution is crucial for distinguishing analytes and their changes over the brewing process. Here, an ester increases and a terpene decreases. Their individual differences are not apparent in the TIC view.

ANALYTE TRENDS

A collection of some analytes that change during the brewing process are shown in Figure 3. The analyte identifications were determined by mass spectral and retention index matching (observed compared to NIST library databases). The heat map shows the relative peak area for each analyte at each point in the process, indicating how that analyte changes through the brewing process. Different compound classes have different behaviors. For example, some esters increase during the boil while others increase during fermentation. Terpenes increase during the boil and then gradually decrease throughout the process. Organic acids and alcohols mostly appear after fermentation. This type of information can help track when specific off-flavors have dropped or when various aroma contributors have reached a desired level.

Name	Sim	R.T. (s)	R.I.	Lib. RI	CAS	Formula
Analytes often tracked during fermentation						
Diacetyl	883	152.17	984.5	979	431-03-8	$C_4H_8O_2$
2,3-Pentanedione	922	231.17	1064.9	1058	600-14-6	$C_5H_8O_2$
Sulfur analytes						
Dimethyl sulfide	967	70.47	794	754	75-18-3	C_2H_6S
Dimethyl disulfide	921	236.94	1070.2	1077	624-92-0	$C_2H_6S_2$
Dimethyl trisulfide	904	535.38	1371.4	1377	3658-80-8	$C_2H_6S_3$
Methional	874	602.90	1452.9	1454		C_4H_8OS
Ester - increase during boil						
Methyl 6-methyl heptanoate	857	504.53	1336.2	1338	2519-37-1	$C_9H_{18}O_2$
Nonanoic acid, methyl ester	848	629.77	1486.3	1491	1731-84-6	$C_{10}H_{20}O_2$
4-Decenoic acid, methyl ester	929	730.21	1620.8	1617	1191-02-2	$C_{11}H_{20}O_2$
Methyl 6-methyloctanoate	890	600.92	1450.4	1482	5129-62-4	$C_{10}H_{20}O_2$
Decanoic acid, methyl ester	845	707.85	1589.8	1593	110-42-9	$C_{11}H_{22}O_2$
Ester - increase during boil and fermentation						
Isobutyl acetate	917	180.33	1017.5	1012	110-19-0	$C_6H_{12}O_2$
Propanoic acid, 2-methyl-, 2-methylpropyl ester	932	257.50	1089.4	1090	97-85-8	$C_8H_{16}O_2$
Propanoic acid, 2-methyl-, hexyl ester	874	503.94	1335.5	1339	2349-07-7	$C_{10}H_{20}O_2$
Acetic acid, heptyl ester	922	532.32	1367.9	1377	112-06-1	$C_9H_{18}O_2$
Ester - increase during fermentation						
Ethyl Acetate	921	101.19	900.2	888	141-78-6	$C_4H_8O_2$
Butanoic acid, ethyl ester	938	203.73	1039.3	1035	105-54-4	$C_6H_{12}O_2$
Hexanoic acid, ethyl ester	950	403.97	1228.8	1233	123-66-0	$C_8H_{16}O_2$
Octanoic acid, ethyl ester	928	585.82	1431.6	1435	106-32-1	$C_{10}H_{20}O_2$
Decanoic acid, ethyl ester	944	739.73	1634.3	1638	110-38-3	$C_{12}H_{24}O_2$
Dodecanoic acid, ethyl ester	873	876.29	1836.5	1841	106-33-2	$C_{14}H_{28}O_2$
1-Butanol, 3-methyl-, acetate	941	289.00	1118.8	1122	123-92-2	$C_7H_{14}O_2$
Acetic acid, 2-phenylethyl ester	908	859.81	1810.6	1813	103-45-7	$C_{10}H_{12}O_2$
Monoterpene						
α-Pinene	906	180.81	1017.9	1028	80-56-8	$C_{10}H_{16}$
Camphene	907	221.87	1056.2	1071	79-92-5	$C_{10}H_{16}$
β-Pinene	927	258.02	1089.9	1112	127-91-3	$C_{10}H_{16}$
β-Myrcene	918	324.67	1152.1	1161	123-35-3	$C_{10}H_{16}$
Limonene	926	350.22	1175.9	1200	138-86-3	$C_{10}H_{16}$
Sesquiterpene						
γ-Muurolene	930	771.05	1678.6	1692	30021-74-0	$C_{15}H_{24}$
Humulene	908	757.79	1659.8	1667	6753-98-6	$C_{15}H_{24}$
Caryophyllene	949	703.58	1584.1	1595	87-44-5	$C_{15}H_{24}$
Terpenoid						
Linalool	860	673.76	1544.2	1547	78-70-6	$C_{10}H_{18}O$
Methyl geraniate	922	778.46	1689	1686	2349-14-6	$C_{11}H_{18}O_2$
Geraniol	853	880.85	1843.7	1847	106-24-1	$C_{10}H_{18}O$
.tau.-Cadinol	868	1070.90	2162.6	2169	5937-11-1	$C_{15}H_{26}O$
Organic Acids						
Acetic acid	911	612.75	1465.2	1449	64-19-7	$C_2H_4O_2$
Hexanoic acid	923	891.99	1861.2	1846	142-62-1	$C_6H_{12}O_2$
Octanoic acid	916	1018.24	2069.7	2060	124-07-2	$C_8H_{16}O_2$
Nonanoic acid	882	1080.90	2180.5	2171	112-05-0	$C_9H_{18}O_2$
n-Decanoic acid	922	1137.61	2285.2	2276	334-48-5	$C_{10}H_{20}O_2$
Alcohols						
Ethanol	927	127.79	944.2	932	64-17-5	C_2H_6O
1-Butanol, 3-methyl-	923	387.48	1211.8	1209	123-51-3	$C_5H_{12}O$
Phenylethyl Alcohol	931	922.86	1910.2	1906	60-12-8	$C_8H_{10}O$
1-Hexanol	880	519.01	1352.7	1355	111-27-3	$C_6H_{14}O$
Phenol	897	980.58	2005.2	2000	108-95-2	C_6H_6O
Aldehyde						
Propanal, 2-methyl-	902	80.57	829	819	78-84-2	C_4H_8O
Butanal, 2-methyl-	831	111.74	917.7	914	96-17-3	$C_5H_{10}O$
Butanal, 3-methyl-	928	113.92	921.3	918	590-86-3	$C_5H_{10}O$
Pentanal	914	150.53	981.8	979	110-62-3	$C_5H_{10}O$
Hexanal	951	248.70	1081.2	1083	66-25-1	$C_6H_{12}O$
Octanal	863	457.16	1283.7	1289	124-13-0	$C_8H_{16}O$
Benzaldehyde	951	655.08	1519.2	1520	100-52-7	C_7H_6O
Benzeneacetaldehyde	934	743.89	1640.2	1640	122-78-1	C_8H_8O
Ketone						
Acetone	872	81.37	831.8	819	67-64-1	C_3H_6O
2-Butanone	898	106.23	908.5	907	78-93-3	C_4H_8O
2-Octanone	936	453.61	1280.1	1287	111-13-7	$C_8H_{16}O$
2-Nonanone	839	545.98	1383.5	1390	821-55-6	$C_9H_{18}O$
2-Decanone	865	630.80	1487.6	1494	693-54-9	$C_{10}H_{20}O$
2-Undecanone	949	710.31	1593.1	1598	112-12-9	$C_{11}H_{22}O$
2-Dodecanone	832	751.65	1651.1	1698	6175-49-1	$C_{12}H_{24}O$
Furans						
Furan	904	78.01	820.1	798	110-00-9	C_4H_4O
Furan, 3-methyl-	883	95.73	881.5	853	930-27-8	C_5H_6O
Furan, 2-methyl-	905	104.67	906	869	534-22-5	C_5H_6O
Furan, 2-ethyl-	936	133.69	953.9	950	3208-16-0	C_6H_8O
Furfural	968	611.74	1463.9	1462	98-01-1	$C_5H_4O_2$
Furan, 3-(4-methyl-3-pentenyl)-	908	571.13	1413.4	1429	539-52-6	$C_{10}H_{14}O$
Aromatic						
o-Cymene	899	433.24	1259	1275	527-84-4	$C_{10}H_{14}$
Benzene, 1,2,4-trimethyl-	918	444.87	1271.1	1283	95-63-6	C_9H_{12}
Nitrogen						
Indole	891	1218.14	2442	2445	120-72-9	C_8H_7N
Pyrrole	913	651.82	1514.8	1514	109-97-7	C_4H_5N

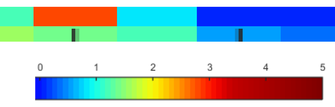
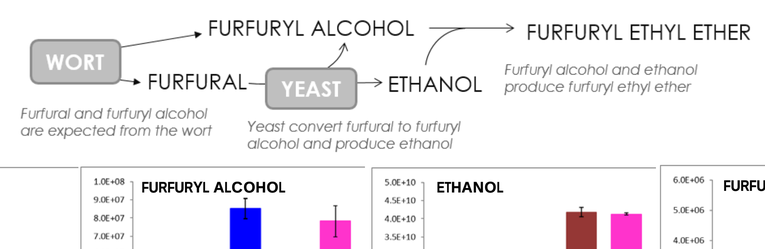


Figure 3. Representative analytes. Identification information is compiled and the heat map indicates relative changes over the brewing process per analyte.

RELATED ANALYTES

This information can also show how analytes may relate to each other. For example, the relationship between furfural, furfuryl alcohol, ethanol, and furfuryl ethyl ether is described in Figure 4. The decreases and increases of individual analyte seem to connect with the expected reactions.



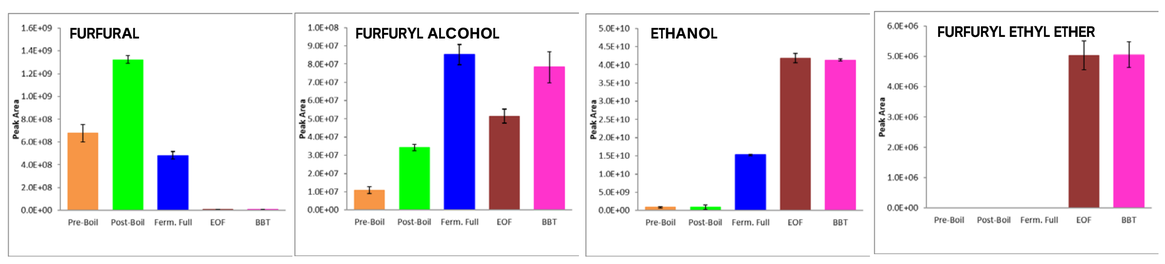


Figure 4. Furfural and furfuryl alcohol, both expected from wort, are observed to increase during the boil. Yeast convert furfural to furfuryl alcohol, and a decrease in furfural along with an increase of furfuryl alcohol is observed after yeast are added at the start of fermentation. Yeast also produce ethanol, which is observed to increase at the start of fermentation. Ethanol and furfuryl alcohol can react with each other to produce furfuryl ethyl ether, which is observed at the end of fermentation along with a corresponding decrease in furfuryl alcohol.

CONCLUSIONS

Many chemical changes occur during the brewing process as raw materials are taken through various stages to yield the finished product. GC-MS is an effective way to determine individual chemicals and to understand the changes they undergo during the brewing process. Hundreds of analytes were detected in these samples and information for representative analytes was presented. Deconvolution was crucial in discerning chromatographic coelutions and provided information on more analytes. Observing changes in the aroma profile provided good insight to the chemical changes occurring throughout the process.

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