Application Note

Instrument: Pegasus® BT

Brewery Process Monitoring with GC-MS

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Key Words: Process Monitoring, HS-SPME, GC-MS, TOFMS, Beer Brewing, Deconvolution

Introduction

Process monitoring by tracking the chemical changes that occur as raw materials are taken through various stages to yield a finished product has the potential to direct process optimization, improve the final product, and improve efficiency of the process. With food and beverage products, the steps of the process and variations in these steps cause changes that are apparent in the associated aroma profile. Monitoring the volatile and semi-volatile analytes that comprise the aroma profile can be accomplished with gas chromatography coupled with mass spectrometry (GC-MS) which is well suited for this type of analysis. In this work, we use GC-MS to probe five points throughout the beer brewing process. Samples were collected pre-boil, post-boil, at the start of fermentation, at the end of fermentation, and from the bright beer tank prior to conditioning. 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 collected on the SPME fiber were subsequently separated from each other as they traveled through the GC column. MS detection provided information for identification and relative quantitation. Observing these types of changes in the aroma profile and connecting them to the steps of the brewing process can provide good insight to the chemical changes occurring throughout the process.

	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. Various points during the brewing process (labeled 1 through 5) were monitored with GC-MS. Representative chromatograms at each processing point are shown. Example analytes that clearly changed during the brewing process are also shown (labeled A through D).

Experimental

Samples were collected at various stages of the brewing process (pre-boil, post-boil, start of fermentation, end of fermentation, and from a tank of bright beer), as indicated in Figure 1. 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. Data for an alkane standard was also acquired to calculate retention indices to filter library search results.

Table 1. GC-TOFMS (*Pegasus* BT) Conditions

Autosampler	LECO LPAL 3
SPME Fiber	DVB/CAR/PDMS fiber (conditioned 5 min pre-injection at 250 °C)
Incubation and Extraction	Incubate 10 minutes and Extract 20 minutes at 35 °C
Gas Chromatograph	**Agilent 7890**
Injection	SPME, 3 min desorption in 250 °C inlet, splitless
Carrier Gas	He @ 1.4 mL/min
Column	Stabilwax, 30 m x 0.25 mm i.d. x 0.25 μm coating (Restek)
Temperature Program	3 min at 40 °C, ramped 10 °C/min to 250 °C, hold 1 min
Transfer Line	250 °C
Mass Spectrometer	**LECO *Pegasus* BT**
Ion Source Temperature	250 °C
Mass Range	33-500 m/z
Acquisition Rate	10 spectra/s

Results and Discussion

An outline of the brewing process and representative chromatograms from the points at which the process was analyzed are shown in Figure 1. The first sample, "Pre-Boil," was collected at the start of the boiling stage of the brewing process. This sample primarily contains the analytes that were extracted from the malt during mashing. The second sample, "Post-Boil," was collected at the end of the boil during which hops were added. Analytes that were extracted from the hops increase while other analytes that degrade or chemically change during boiling are observed to decrease compared to the "Pre-Boil" sample. The next sample, "Start of Fermentation (SOF)" was taken at the start of fermentation when yeast were added and the fourth sample, "End of Fermentation (EOF)," was collected at the end. Many changes occur during this stage of the process that can be attributed to yeast activity and other chemical reactions. The final sample, "Bright Beer Tank (BBT)," was collected after yeast were no longer in suspension. Hundreds of analytes including esters, terpenes, terpenoids, organic acids, alcohols, aldehydes, ketones, furans, aromatics, nitrogen-containing, and sulfur-containing analytes were detected across these samples. Many differences in the samples were readily apparent, as can be seen in the total ion chromatograms (TIC) in Figure 1, indicating significant chemical changes over the brewing process.

Information for four analytes (labeled A-D) with changes clearly visible in the TIC is shown in Figure 1. Hexanal is an aldehyde from the malt that is observed at highest levels in the "Pre-Boil" sample that then decreases during the boiling stage. β-myrcene is a terpene that is extracted from the hops. It is observed at highest levels in the "Post-Boil" sample with a gradual decline through the remaining stages of the process. Ethanol, a product of yeast activity, was observed to increase during fermentation after the yeast were added. Ethyl octanoate is an ester that increases during fermentation that can likely be attributed to yeast activity. These changes are all clearly apparent in the TIC, but other important differences may be obscured by coelutions and hidden in the TIC view. An example of this is shown in Figure 2. An ester, isobutyl acetate, and a terpene, α-pinene chromatographically coelute. These analytes appear as a single peak in the TIC, but can be clearly distinguished by plotting extracted ion chromatograms (XICs) unique to each analyte. If all samples are overlaid, there appears to be no trend in this peak at this retention time, but the XICs reveal that the ester increases while the terpene decreases. LECO's deconvolution software handled these coeluting analytes in an automated way and was crucial for determining their trends.

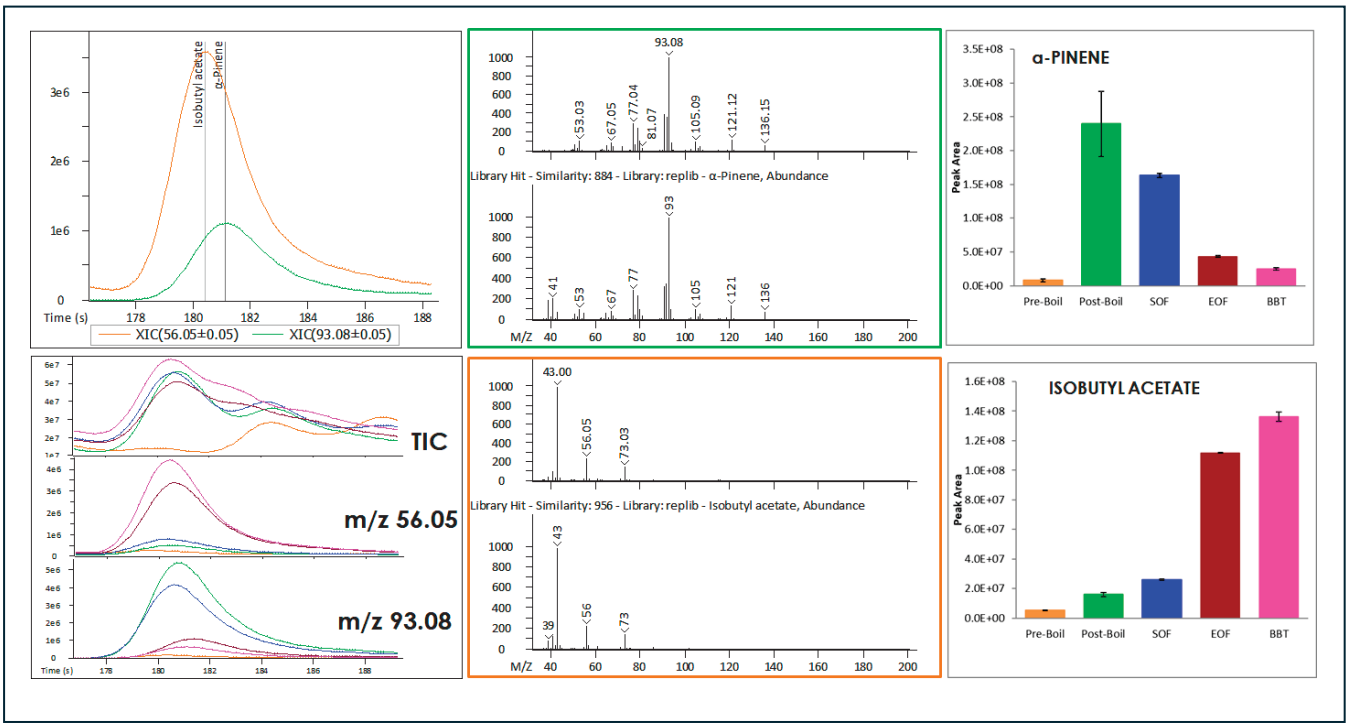

Figure 2. The trends for some analytes may be obscured by coelutions in the TIC view. In these cases, deconvolution is crucial for distinguishing analytes and their changes over the brewing process. The individual differences are not apparent in the TIC view, but deconvolution reveals that the ester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

Additionally, tracking specific chemicals can help to show the chemical reactions that may be occurring through the process. For example, the relationship between furfural, furfuryl alcohol, ethanol, and furfuryl ethyl ether is described in Figure 3. Furfural and furfuryl alcohol are expected from wort and were both observed to increase during the boil. Yeast can convert furfural to furfuryl alcohol and a decrease in furfural along with an increase of furfuryl alcohol was observed after yeast were added at the start of fermentation. Yeast also produce ethanol, which was observed to increase at the start of fermentation. Ethanol and furfuryl alcohol can then react with each other to produce furfuryl ethyl ether, which was observed at the end of fermentation along with a corresponding decrease in furfuryl alcohol.

These analytes and a collection of others that were observed to change during the brewing process are shown in Table 2. 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 changed through the brewing process. Different compound classes have different behaviors and analytes within a compound class may also have different trends. 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 undesired notes have dropped or when various aroma contributors have reached a desired level.

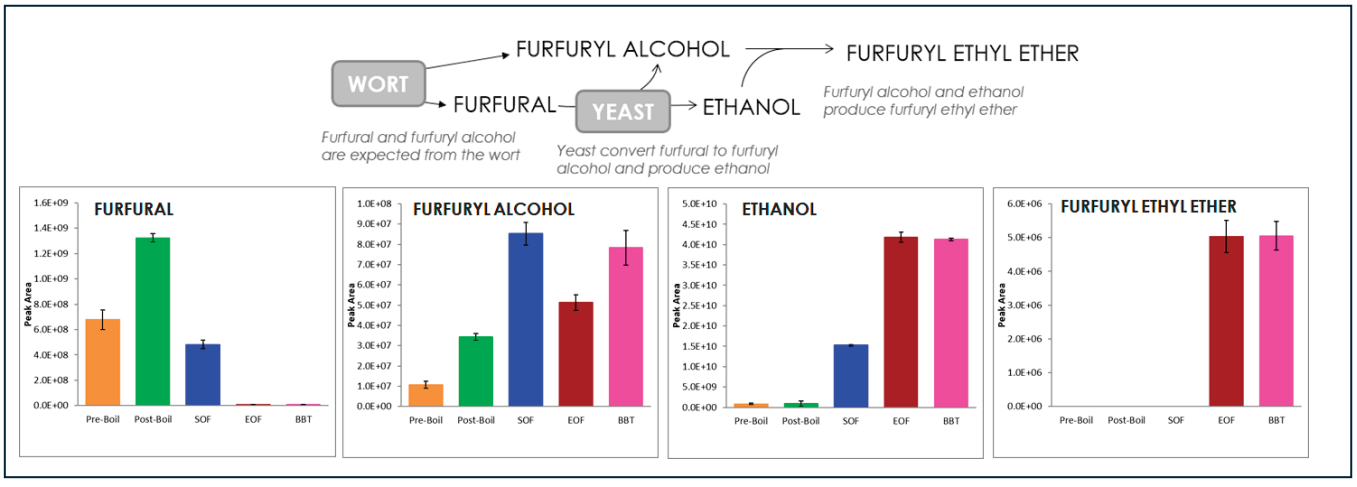

Figure 3. The relationship between furfural, furfuryl alcohol, ethanol, and furfuryl ethyl ether is described. The decreases and increases of the

Table 2. Identification information and relative trends (heat map) for representative analytes indicate changes over the brewing process per analyte.

Name	Sim	R.T. (s)	R.I.	Lib. RI	CAS	Formula	Pre-Boil	Post-Boil	SOF	EOF	BBT
Analytes often tracked during fermentation											
Diacetyl	883	152.17	984.5	979	431-03-8	$C_4H_6O_2$					
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					

Conclusion

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 identify and track 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. Many trends were observed. For example, some analytes from the malt were observed to decrease during the boil while analytes from the hops were observed to increase during the boil. Ethanol was observed to increase during fermentation. Some esters increased during the boil while others increased during fermentation. Deconvolution was crucial in discerning chromatographic coelutions and provided better information for more analytes. Observing changes in the aroma profile provided significant insight to the chemical changes occurring throughout the process.

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