

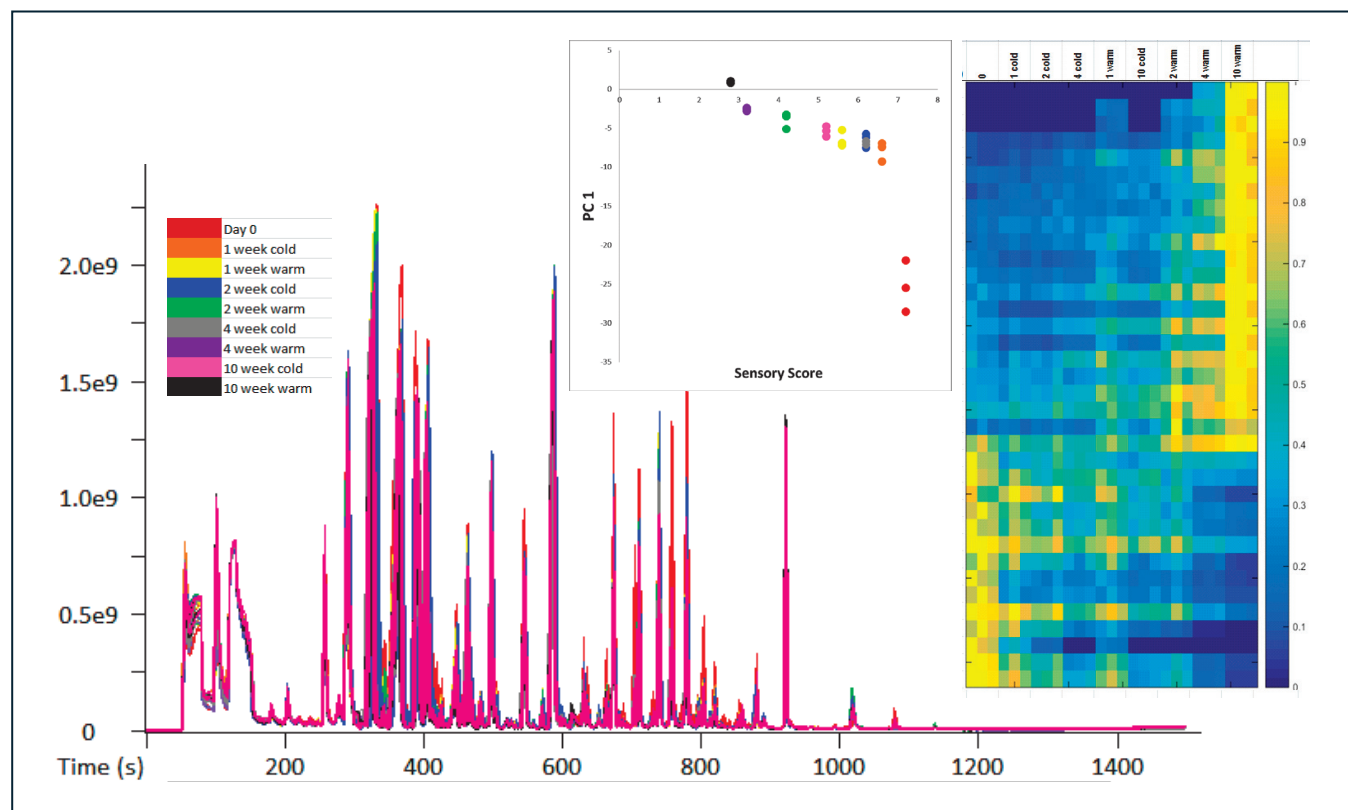
**Instrument: Pegasus BT<sup>®</sup>****Correlation of Chemical and Sensory Data to Track Aging of an American IPA Beer at Different Storage Temperatures**

LECO Corporation; Saint Joseph, Michigan USA

Key Words: Beer, Shelf-Stability, Aroma Profile, Sensory Analysis, HS-SPME, GC-MS, Deconvolution, PCA, Time-of-Flight

**Introduction**

Shelf stability and how products change with age and storage condition is an important topic to both manufacturers and consumers. Understanding changes in the aroma profile over time can help to understand the shelf life of that product and possibly how it can be extended. Gas chromatography coupled to time-of-flight mass spectrometry (GC-TOFMS) is well-suited for investigating shelf stability applications because it provides non-targeted aroma profile characterization. Sensory analysis is also common for shelf-stability and general product quality applications as consumer-detectable changes are readily identified. These are complementary analytical techniques and using them together is beneficial. The sensory analysis can provide important context to understand the observed chemicals and the chemical analysis can help explain the sensory observations. In this work, we combine chemical analysis with sensory analysis to investigate the shelf stability of bottled beer as it relates to storage temperature. A time course series of bottled beer samples were collected so that on the day of analysis a fresh beer and beer at ages 1, 2, 4, and 10 weeks of age were available for analysis. Over the sample collection time frame, one set of samples was stored at room temperature (68-70 °F) and another set was stored under refrigeration (34-36 °F). An expert panel at a collaborating brewery performed the sensory analysis. Chemical analysis that combined headspace solid phase micro-extraction (HS-SPME) with GC-MS was performed at LECO. The sensory analysis provided overall freshness and quality scores for each sample, while the non-targeted GC-MS analysis provided comprehensive aroma profile chemical data. These data were mined for specific analytes of interest and were also reviewed to discover inherent trends and differences in the aroma profile that correlated with the sensory analysis results. We combined the data from each analysis to better understand the relationship of storage condition and shelf life, and to connect chemical changes to the sensory observations.



**Figure 1. Overlaid chromatograms from all samples are shown. A plot showing the correlation of the sensory scores with the PC1 scores from the chemical analysis shows good agreement. The heat map highlights peak areas of analytes that correlate with sensory score.**

## Experimental

**Samples:** A time course series of an American IPA was acquired by collecting bottles directly from the packaging line at a brewery over a 10 week period. Bottles were collected 10, 4, 2, and 1 weeks ahead of analysis as well as on the day of analysis. The bottles were stored on-site at the brewery with one set under refrigeration (maintained between 34-36 °F, cold), and the other set at room temperature (maintained between 68-70 °F, warm).

**Sensory Analysis:** Sensory analysis was performed by an expert panel of five tasters at the brewery. The panel was comprised of individuals from the quality and brewing departments that had undergone comprehensive training on beer oxidation, and were very familiar with the brand being analyzed. Each sample was scored by the panel based on the scoring system outlined in Table 1.

**Table 1. Sensory Scoring System for Freshness Rating Scale**

Score	Description
8	Equivalent to fresh product with full flavor profile present
7	Some flavor profile has diminished, but no oxidation present
6	Diminished flavor profile, but no oxidation present
5	Beginning signs of aging with some flavor profile present
4	Moderate signs of aging with some flavor profile present
3	Extreme signs of aging with little flavor profile present
2	Extreme signs of aging, no longer resembles brand
1	Extreme signs of aging and change in appearance, no longer resembles brand
0	Infection, wrong brand, or not consumable

**Chemical Analysis:** The chemical analysis was performed with HS-SPME coupled to LECO's Pegasus BT GC-MS. For each sample, 5 mL of beer were pipet into a 20 mL glass vial and sealed with a septum cap. The HS-SPME procedure was performed by LECO's L-PAL3 Autosampler. The samples were incubated for 10 min at 35 °C and then extracted with a DVB/CAR/PDMS fiber (Supelco) for 20 min at the same temperature. Immediately after extraction, the SPME fiber was exposed for 3 minutes in the GC-inlet maintained at a temperature of 250 °C in splitless mode. The fiber was conditioned for 5 minutes at 250 °C between each sample. All samples were analyzed in triplicate with the method conditions listed in Table 2. Data for an alkane standard was also collected with the same methods, allowing for Retention Index determinations.

**Table 2. GC-TOFMS (Pegasus BT) Conditions**

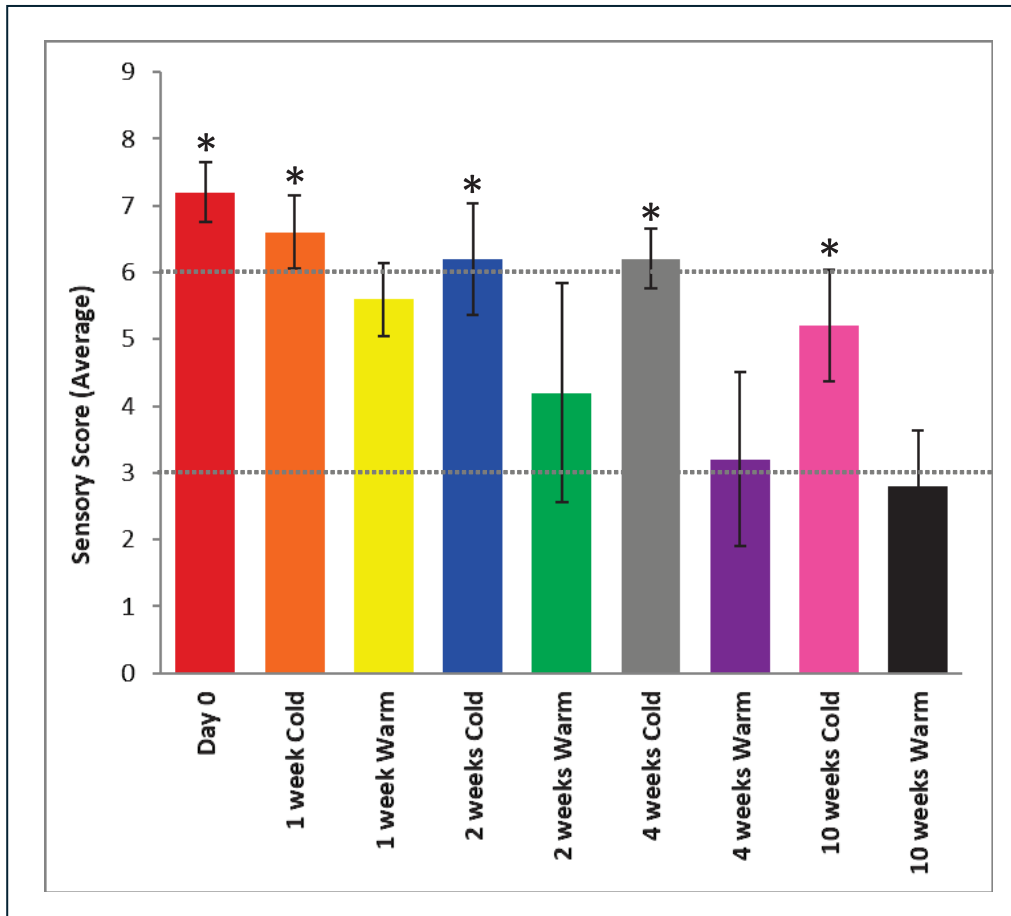
<b>Gas Chromatograph</b>	<b>Agilent 7890 with LECO L-PAL 3 Autosampler</b>
Injection	SPME, 3 min desorption in 250 °C inlet
Carrier Gas	He @ 1.4 mL/min, Constant Flow
Column One	Stabilwax, 30 m x 0.25 mm i.d. x 0.25 µm coating (Restek)
Temperature Program	3 min at 40 °C, ramped 10 °C/min to 250 °C, hold 1 min
Transfer Line	250 °C
<b>Mass Spectrometer</b>	<b>LECO Pegasus BT</b>
Ion Source Temperature	250 °C
Mass Range	33-500 m/z
Acquisition Rate	10 spectra/s

**Data Analysis:** The GC-MS data were processed with LECO's ChromaTOF® brand software that includes deconvolution as part of the automated peak finding. Peak areas were determined with the integration of a single m/z per analyte. Identifications are tentative, but determined by spectral matching to NIST library databases, and by retention index matching of calculated values based on the alkane standard compared to NIST database values. Principal Component Analysis (PCA) was performed on the tabulated peak areas in MatLab.

## Results and Discussion

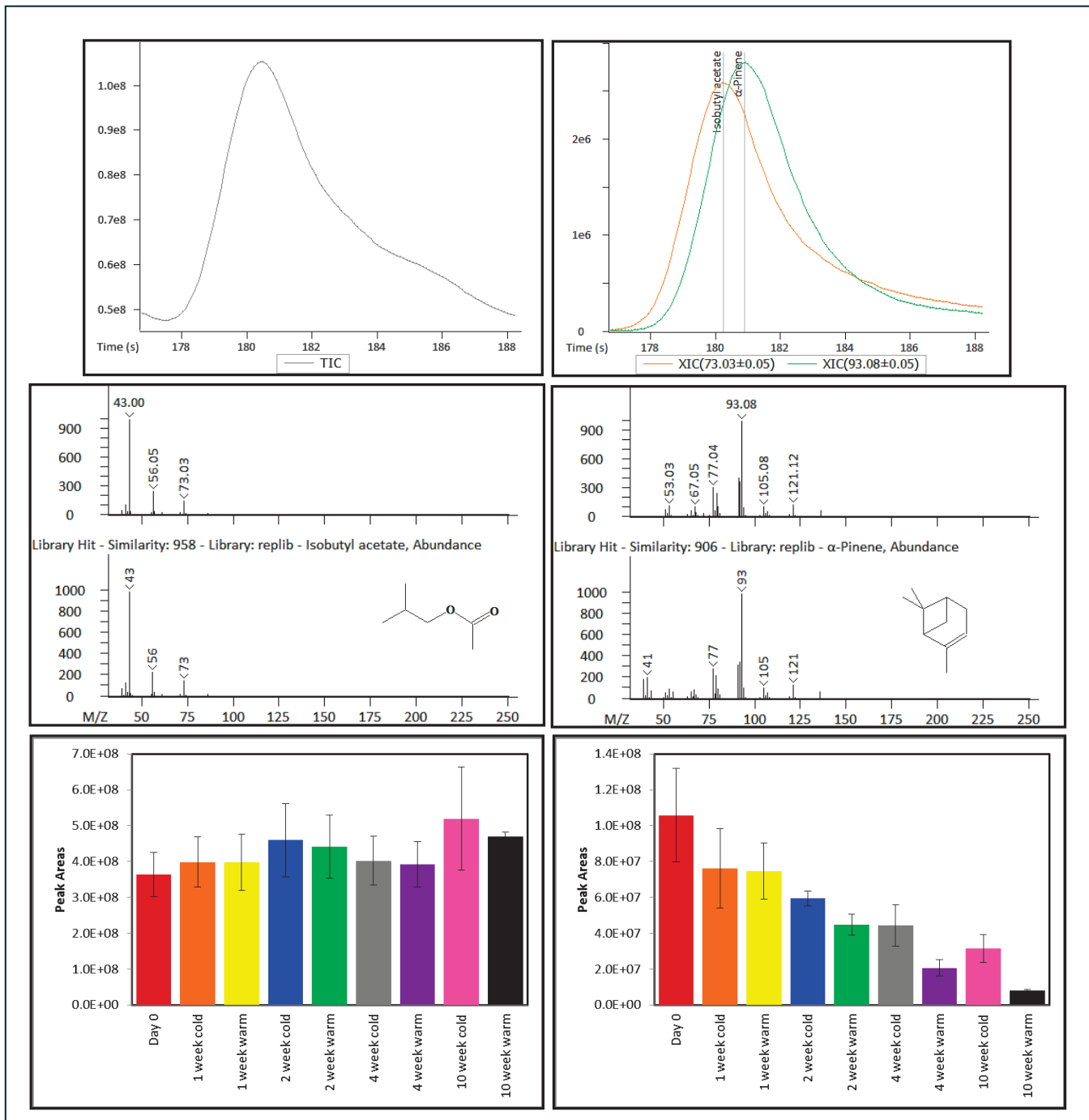
**Sensory Analysis Results:** Each sample was scored by the sensory panel. As described in Table 1, higher scores indicated freshest samples. Scores between 8 and 6 showed decreased aroma, but no signs of aging; scores between 6 and 3 showed more significant decline in aroma along with increased signs of aging; and scores below 3 exhibited severe signs of aging and had little aroma remaining. The panelist's scores are shown in Figure 2. These results indicate that the beer quality decreases with age and does so more rapidly when stored at room temperature than when refrigerated. Under refrigeration (asterisks, Figure 2), the samples show decreased aroma profile, but do not show signs of aging in the first 4 weeks of storage.

The week 4 sensory score is 6.2, which has decreased aroma, but no signs of aging. The week 10 sensory score is 5.2, which has beginning signs of aging along with the decreased aroma. With room temperature storage, however, signs of aging are present after only 1 week (no asterisks, Figure 2). The sensory score at 1 week is 5.6 (similar to 10 weeks of refrigerated storage). The sensory scores of room temperature storage continue to decrease to a score of 2.6 at 10 weeks, which indicates a sample with extreme signs of aging that is no longer representative of the brand.



**Figure 2. Sensory analysis results. Freshness shows a general decline with age with the change occurring more rapidly at room temperature storage than at refrigerated storage.**

**Chemical Analysis Results:** Each beer sample was also subjected to chemical analysis with HS-SPME coupled to GC-MS. Representative TIC chromatograms for the samples are overlaid in Figure 1. ChromaTOF provides automated peak finding that uncovered hundreds of analytes in these chromatograms. Many analytes are chromatographically isolated, but coelution is common with complex samples. With full mass range acquisition, deconvolution can mathematically resolve the overlapping regions and provide information on each unique analyte in many of these instances. An example is shown in Figure 3. There appears to be one peak around 180 s (with a likely coelution in the tail around 185 s) in the TIC view. However, the automated peak finding with deconvolution determined there were two analytes that elute at ~180 s. The distinct peak shapes were observed by plotting XICs for individual masses that are unique to each analyte, m/z 73.03 and m/z 93.08. In addition to peak profiles, the software isolates pure spectral information that can be matched to libraries for identification. In this example, the first analyte is identified as isobutyl acetate and the second is alpha-pinene with similarity scores of 958 and 906, respectively. Retention index information supports these identifications with calculated RI values of 1017 and 1018 compared to the NIST values of 1012 and 1028 for the ester and terpene, respectively. Once identified, the aroma contributions of each can be determined. The ester is described as sweet, fruity, ethereal, banana, and tropical; and the terpene is described as fresh, camphor, sweet, pine, earthy, and woody. The trends of these analytes across the sample set are also shown in Figure 3. The ester does not seem to change related to age or storage temperature, but the terpene does. The terpene has highest levels in the freshest sample and decreases with age, more rapidly at room temperature than when refrigerated. This particular analyte shows similar trends in peak area as were observed in the overall sensory score. It would have been obscured without deconvolution.



**Figure 3. Deconvolution example. As ester and a terpene coelute, but are mathematically resolved with deconvolution, allowing for the identification of each and the determination of their relative trends across the sample set.**

Hundreds of other analytes were determined with the fully automated peak finding tools, including hydrocarbons, esters, terpenes, alcohols, aldehydes, ketones, sulfur-containing analytes, nitrogen-containing analytes, etc. The peak areas for over 350 analytes were compiled and compared across the sample set to observe trends for individual analytes. Some analytes were consistent across the sample set, like the ester shown in Figure 3, or had variability that was not related to age or storage condition. Other analytes, however, did trend with time and storage condition, similar to the terpene in Figure 3. Some representative examples are shown in Figure 4. L- $\beta$ -pinene, a terpene, and methyl ester benzeneacetic acid, an ester, were both observed to decrease with age. In both instances, the decrease occurred more rapidly with room temperature storage. Other analytes were observed to increase with age and temperature. For example, furfural, a Maillard reaction product, and 2-methyl propanal, a Strecker aldehyde, both increased with age and did so more rapidly at room temperature storage.

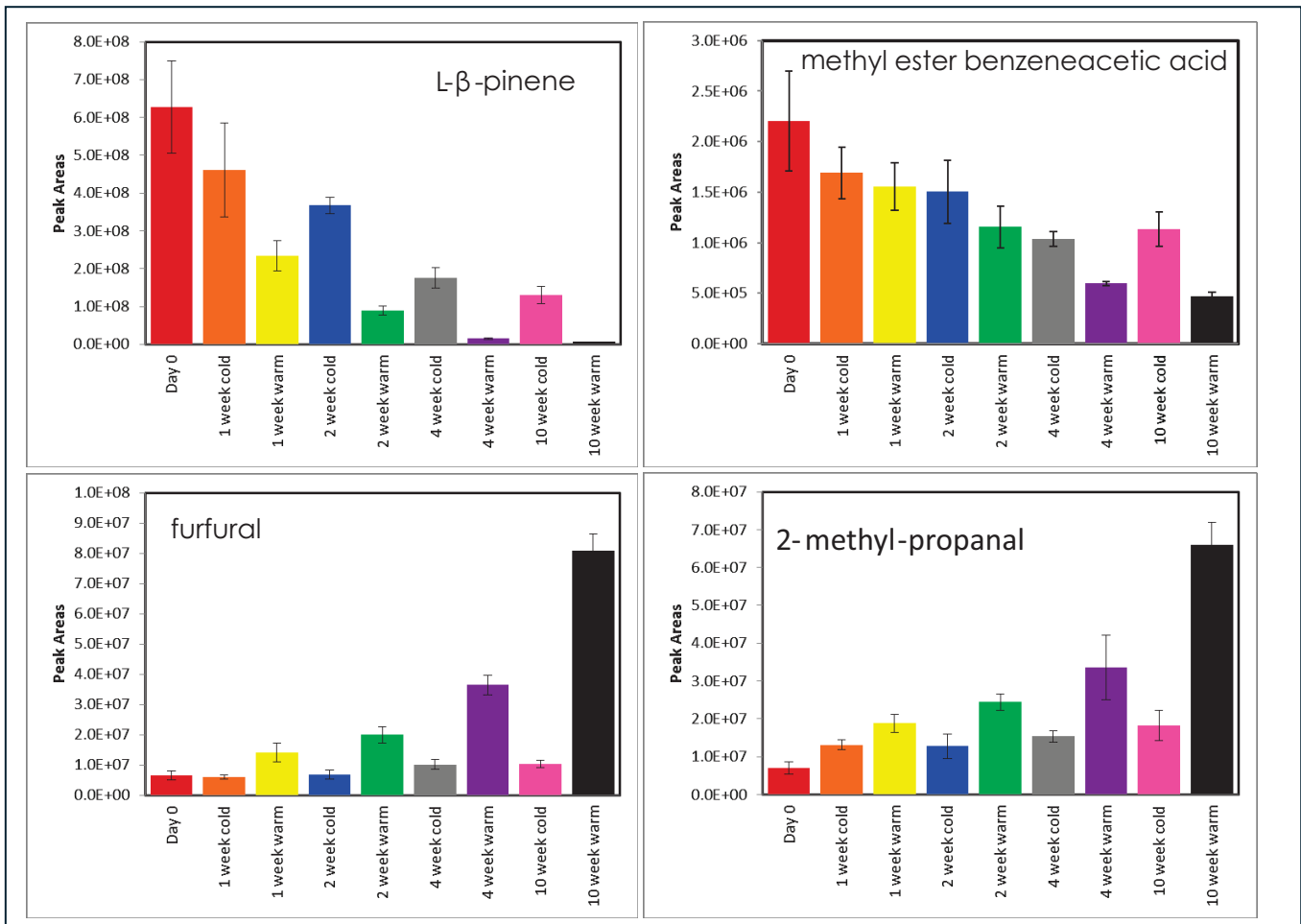


Figure 4. Representative analyte trends.

In addition to investigating at the individual analyte level, overall chemical trends were also explored with Principal Component Analysis (PCA). PCA determines similarities and differences between samples based on the inherent variability in the complete data. All 350 chemical compounds were used as variables and the associated scores plot is shown in Figure 5. Each sample is represented as a data point in this plot and the proximity of the data points indicates the similarities between the samples when all variables are considered together. The replicates for each sample group together and some age and storage condition dependence is apparent. The scores on PC1, in particular, track with the age and storage condition as shown in Figure 5B, where the inverted PC1 score is plot vs sample. This plot parallels the trends in the sensory data, shown in Figure 2. This result indicates that the largest source of variation in the chemical data relates to the trends and variation that were determined by the sensory panel.

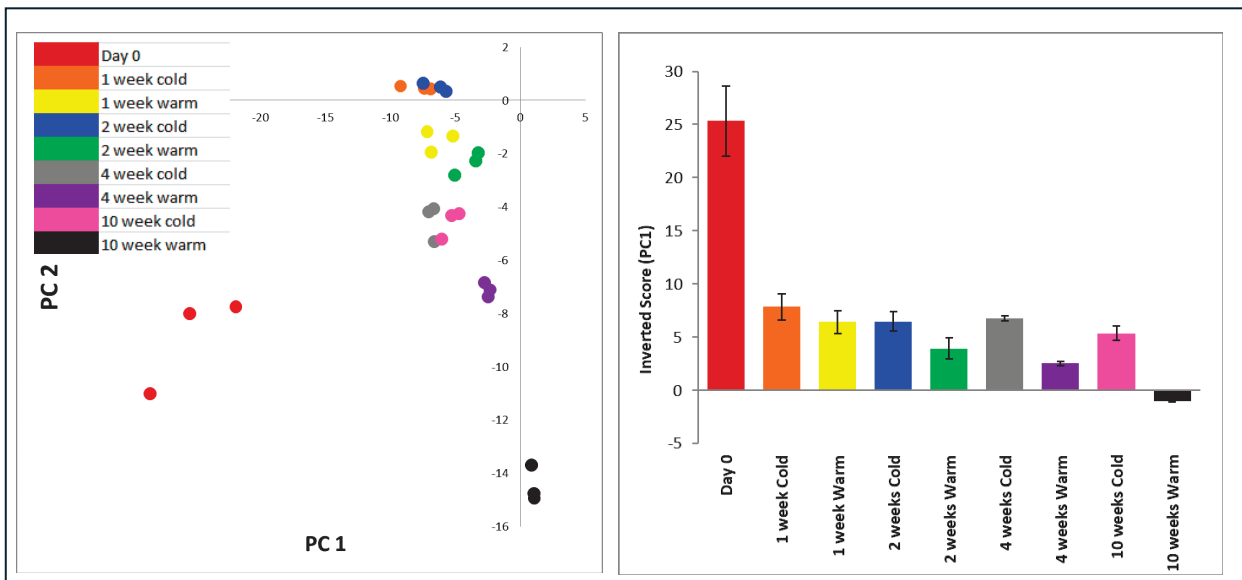


Figure 5. PCA scores plot. PC1 (shown inverted), in particular, parallels the trends picked up by the sensory panel (plotted in Figure 2).

**Correlation of Analytical Results:** To further explore the parallels between the chemical and sensory data, the overall chemical score (PC1) was compared to the overall sensory score, shown in the inset of Figure 1. The trend between the two analytical approaches is apparent. Some deviation can be observed in the freshest samples, which suggests that there were chemical changes at the earliest time point that did not impact the sensory score. The corresponding loadings point to the chemicals most responsible for the differences in the samples. The peak areas for each analyte were also plot against the overall sensory score and correlations were calculated for every analyte, resulting in a list of 36 analytes that trend together. A heat map that shows the area trends for these 36 analytes is shown on the inset of Figure 1. Analyte details are tabulated in Table 3. Many of the analytes that are at the top of the heat map (that negatively correlated with sensory scores) are things like furans, Maillard reaction products, and Strecker aldehydes. Some of the associated aroma characteristics for these analytes are bready, animal, meaty, and gassy. The analytes at the bottom of the heat map are those that are positively associated with sensory score and are analytes like esters and terpenes. These have odor descriptors like fruity, honey, herbal, floral, and green.

**Table 3. Analytes that Correlate (Figure 1)**

	Name	iR (s)	Sim	CAS	Formula	RI (obs)	RI (Lib)
1	butanedioic acid, monomethyl ester	213.3	713	3878-55-5	C <sub>5</sub> H <sub>8</sub> O <sub>4</sub>	1053.4	
2	geranyl vinyl ether	643.3	783		C <sub>12</sub> H <sub>20</sub> O	1503.4	
3	unknown	92.0				868.7	
4	furfural	612.0	943	98-01-1	C <sub>5</sub> H <sub>4</sub> O <sub>2</sub>	1464.2	1462
5	unknown	884.3				1849.1	
6	furan, 2,5-dimethyl-	134.1	873	625-86-5	C <sub>6</sub> H <sub>8</sub> O	954.7	939
7	propanal, 2-methyl-	80.7	903	78-84-2	C <sub>4</sub> H <sub>8</sub> O	829.6	819
8	furfuryl ethyl ether	459.4	861		C <sub>7</sub> H <sub>10</sub> O <sub>2</sub>	1286	
9	furan, 3-methyl-	95.5	923	930-27-8	C <sub>5</sub> H <sub>6</sub> O	880.7	853
10	furan, 2-ethyl-5-methyl-	197.6	855	1703-52-2	C <sub>7</sub> H <sub>10</sub> O	1033.6	1015
11	cadalene	1100.6	888	483-78-3	C <sub>15</sub> H <sub>18</sub>	2216.3	2223
12	naphthalene, 1,2-dihydro-1,1,6-trimethyl-	810.8	782	30364-38-6	C <sub>13</sub> H <sub>16</sub>	1736.8	1732
13	1-(3-methyl-2H-pyrazol-4-yl) ethanone	222.6	805		C <sub>6</sub> H <sub>8</sub> N <sub>2</sub> O	1056.9	
14	unknown	614.9				1467.8	
15	myrtenyl angelate	499.8	767	138530-45-7	C <sub>15</sub> H <sub>22</sub> O <sub>2</sub>	1330.7	
16	α-coralene	1009.7	837	20129-39-9	C <sub>15</sub> H <sub>20</sub>	2055.1	2060
17	indole	1218.3	823	120-72-9	C <sub>8</sub> H <sub>7</sub> N	2442.3	2445
18	unknown	547.4				1385.2	
19	1,6,10-dodecatrien-3-ol,3,7,11-trimethyl-(E)	996.9	842	7212-44-4	C <sub>15</sub> H <sub>26</sub> O	2033	2033
20	unknown	526.1				1360.9	
21	unknown	286.1				1116.1	
22	α-phellandrene	315.5	920	99-83-2	C <sub>10</sub> H <sub>16</sub>	1143.5	1167
23	methyl salicylate	833.5	890	119-36-8	C <sub>8</sub> H <sub>8</sub> O <sub>3</sub>	1770.7	1765
24	unknown	822.4				1754.2	
25	lavandulol acetate	820.0	861	25905-14-0	C <sub>12</sub> H <sub>20</sub> O <sub>2</sub>	1750.5	
26	heptanoic acid, methyl ester	424.9	752	106-73-0	C <sub>8</sub> H <sub>16</sub> O <sub>2</sub>	1250.5	1284
27	benzeneacetic acid, methyl ester	823.9	900	101-41-7	C <sub>9</sub> H <sub>10</sub> O <sub>2</sub>	1756.4	1750
28	ethane, 1,1,1-trimethoxy-	464.6	704	1445-45-0	C <sub>5</sub> H <sub>12</sub> O <sub>3</sub>	1291.4	
29	α-pinene	180.9	906	80-56-8	C <sub>10</sub> H <sub>16</sub>	1018	1028
30	geranyl isobutyrate	834.5	839	2345-26-8	C <sub>14</sub> H <sub>24</sub> O <sub>2</sub>	1772.3	1819
31	disulfide, dimethyl	236.8	916	624-92-0	C <sub>2</sub> H <sub>6</sub> S <sub>2</sub>	1070.1	1077
32	4-hexen-1-ol, 2-methylpropionate	557.7	882		C <sub>10</sub> H <sub>18</sub> O <sub>2</sub>	1396.9	
33	L-β-pinene	258.4	894	18172-67-3	C <sub>10</sub> H <sub>16</sub>	1090.3	
34	propanoic acid, 2-methyl-, octyl ester	668.1	826	109-15-9	C <sub>12</sub> H <sub>24</sub> O <sub>2</sub>	1536.6	1543
35	propanoic acid, hexyl ester	502.2	792	2445-76-3	C <sub>9</sub> H <sub>18</sub> O <sub>2</sub>	1333.4	1349
36	propanoic acid, 2-methyl-, hexyl ester	504.5	925	2349-07-7	C <sub>10</sub> H <sub>20</sub> O <sub>2</sub>	1336.1	1339

## Conclusion

In this work, we aimed to investigate the impact of storage temperature on the shelf life of an American IPA Beer. Chemical and sensory data were combined and we observed that freshness generally declines with age and that this degradation occurs at a faster rate with higher temperature storage compared to lower temperature storage. The sensory analysis gave good information on the quality of the samples overall, and provided important context to probe the chemical data. The chemical analysis uncovered specific analytes that were related to the sensory observations and that correlate with sensory score. We also demonstrated an effective way to connect data from these complementary analysis techniques. Using both data sets together, we were able better understand these samples than we would have with either data set independently.



**LECO Corporation** | 3000 Lakeview Avenue | St. Joseph, MI 49085 | Phone: 800-292-6141 | 269-985-5496  
info@leco.com • www.leco.com | ISO-9001:2015 Q-994 | LECO is a registered trademark of LECO Corporation.  
Pegasus, ChromaTOF are registered trademarks of LECO Corporation.