Investigation of Aging in Beer Using a New Gas Chromatography Time-of-Flight Mass Spectrometry Benchtop System

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Introduction

Gas chromatography paired with time-of-flight mass spectrometry (GC-TOFMS) was used to investigate the aging process of beer to determine time course trends for individual analytes within the aroma profile. This type of non-targeted chemical analysis can provide more insight than targeted approaches, and is a good complement to other traditional analyses, such as sensory panels. Rather than specifying target analytes in advance, analytes of interest can be determined from the data. A time course series of samples was prepared by artificially accelerating aging with storage at elevated temperature for various lengths of time. One day of storage at 40 °C was used to represent one month of storage under appropriate conditions. This protocol was used to produce a time course series of beer samples effectively aged an additional 0, 1, 2, 3, 4, 5, 6, 8, 12, and 20 months. The samples were analyzed in triplicate using headspace solid-phase micro-extraction (HS-SPME) to collect and concentrate volatile and semi-volatile analytes in the headspace of each sample. Chemical analysis was then accomplished with a new commercially-available GC-TOFMS benchtop system. The data were analyzed for specific targets and mined for previously unknown trends and differences. Identification information and relative quantitative information were determined for over 300 analytes. From these data, specific time course trends were observed for some analytes, providing a better understanding of the samples and the aging process.

Methods

Samples and Aging Experiment: A commercially-available IPA was purchased and artificially aged with storage at elevated temperatures. A previously reported protocol calibrated that 1 day of storage at 40 °C was comparable to 1 month of storage under appropriate conditions. (Ref: Marques, ASBC A30. Chicago, IL. June 2014) That information was used to generate a time course set of samples by storing samples at 40 °C for 0, 1, 2, 3, 4, 5, 6, 8, 12, or 20 days. This sample preparation is diagramed in Figure 1.

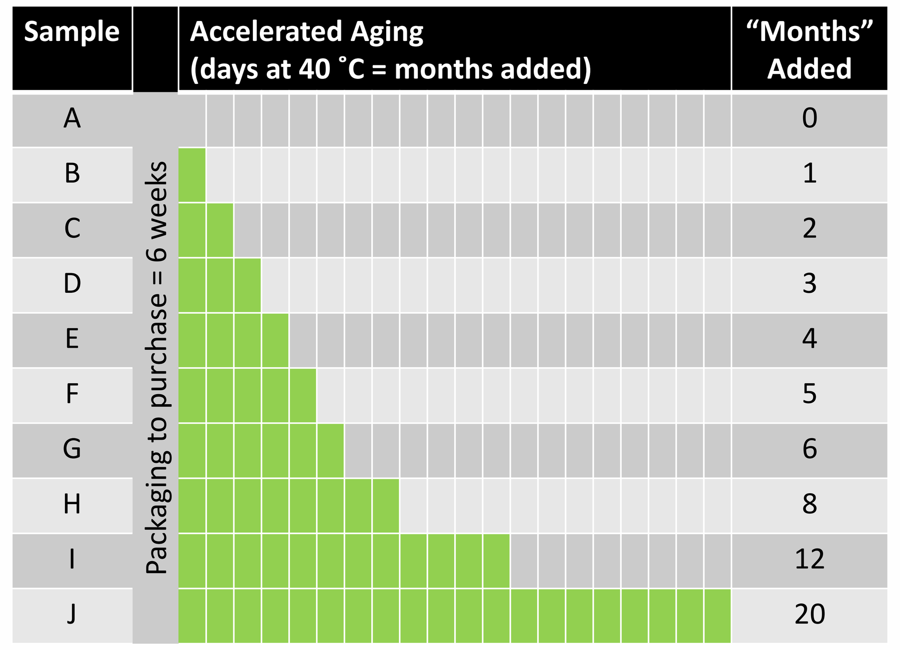

Figure 1. Diagram of forced aging experiment.

Analytical analysis: For each sample, 5 mL of beer were pipetted into a 10 mL glass vial. The samples incubated for 10 min at 60 °C. Extraction was performed with a DVB/CAR/PDMS fiber (Supelco) for 20 min also at 60 °C prior to analysis by GC-TOFMS. Instrument conditions are listed in Table 1

Table 1. GC-TOFMS Instrument Conditions

Gas Chromatograph	**Agilent 7890 with LECO L-PAL3 Autosampler**
Injection	3 min desorption in 250 °C inlet
Carrier Gas	He @ 1.0 ml/min, Constant Flow
Column	Stabilwax, 30 m x 0.25 mm i.d. x 0.25 µm coating (Restek)
Oven Program	Hold 4 min at 35 °C, ramp 5 °C/min to 180 °C, ramp 10 °C/min to 220 °C and hold 5 min
Transfer Line	250 °C
Mass Spectrometer	**LECO Pegasus® BT**
Ion Source Temp	250 °C
Mass Range	35-650 m/z
Acquisition Rate	10 spectra/s

GC-TOFMS

HS-SPME with GC-TOFMS offered an overview of the aging process. These samples have significant complexity and hundreds of peaks were observed in the aroma profile. A variety of analytes including terpenes, esters, aromatic compounds, acids, aldehydes, ketones, heterocyclic compounds, alcohols, etc. were identified through library matching and retention index. TIC chromatograms are overlaid for all time points in Figure 2. Examples of the trends observed for a variety of targeted esters and terpenes are shown in Figures 3 and 4, respectively. Not all analytes from a given compound class displayed the same trend.

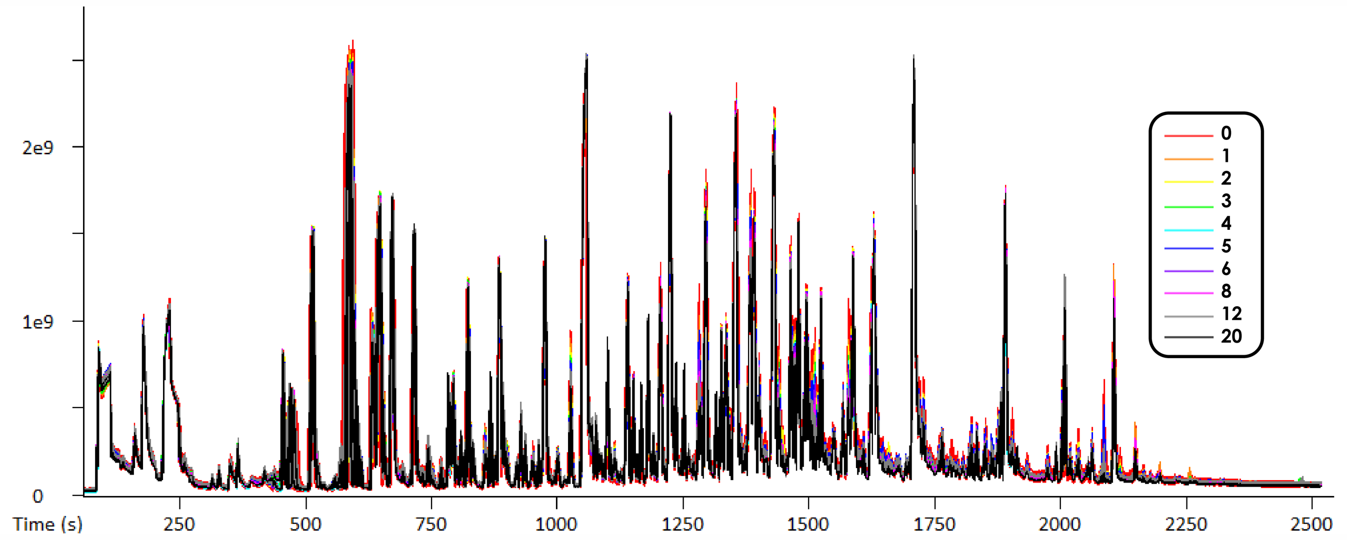

Figure 2. TIC chromatograms are overlaid for all time points in the aging experiment.

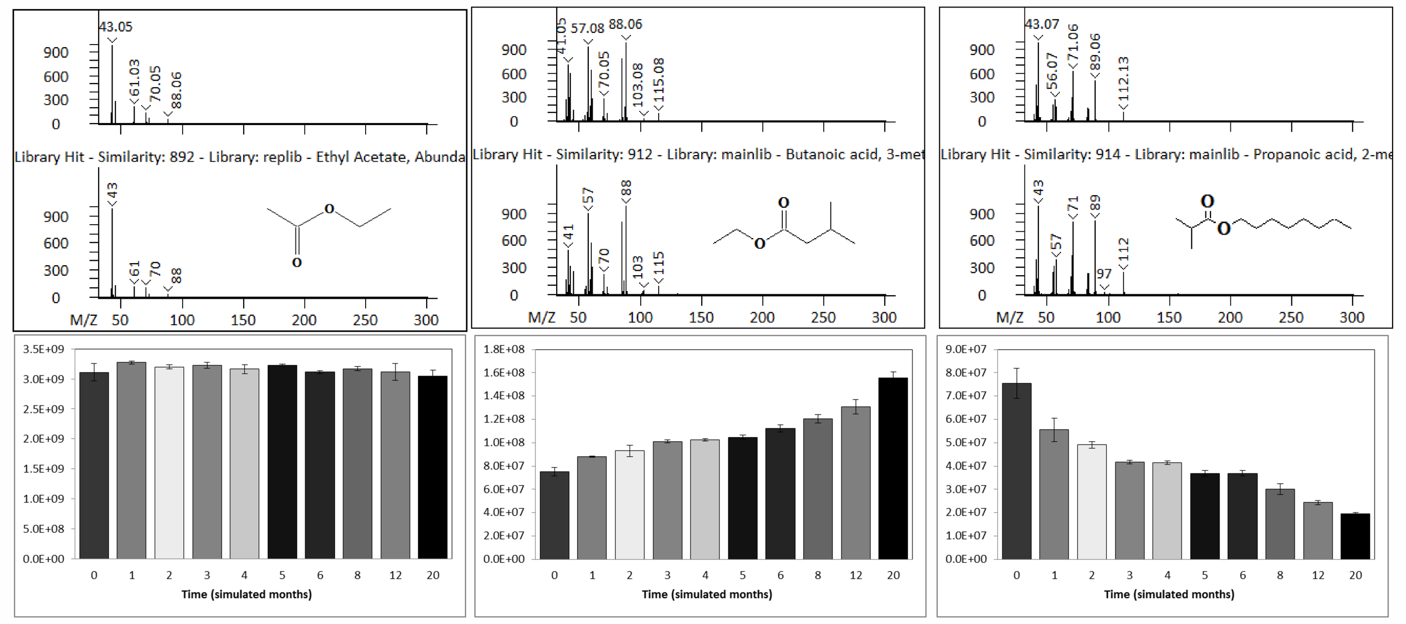

Figure 3. Examples of some of the trends observed for various esters.

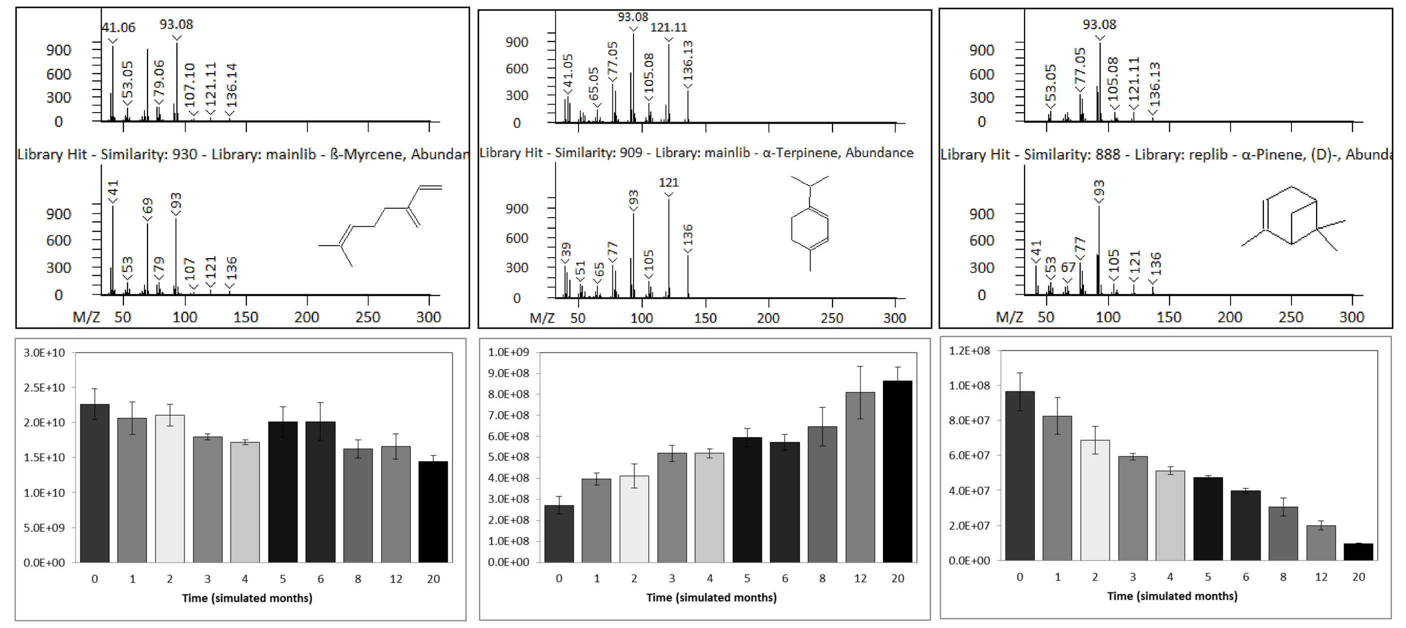

Figure 4. Examples of some of the trends observed for various terpenes.

Non-Target Analysis

General peak finding was performed and peak area information for the peaks was aligned between the samples. This resulted in over 300 analytes compiled across the time course. To determine differences and trends within these analytes, a t-test was calculated between peak area data from time 0 and time 20. This highlighted analytes that differed from the beginning to the end of the time course. Regression statistics were then used to distinguish those that were trending with the time course rather than exhibiting random variation between time points. 108 analytes were found to differ between the beginning and the end of the time course and to correlate with aging. Those analytes are summarized in the heat map, shown in Figure 5. Each row is an analyte, and each column is a time point. The color scale shows relative intensity, so time course trends for each analyte can be observed by the color change across a row. Representative examples are shown in Figure 6 and Table 2.

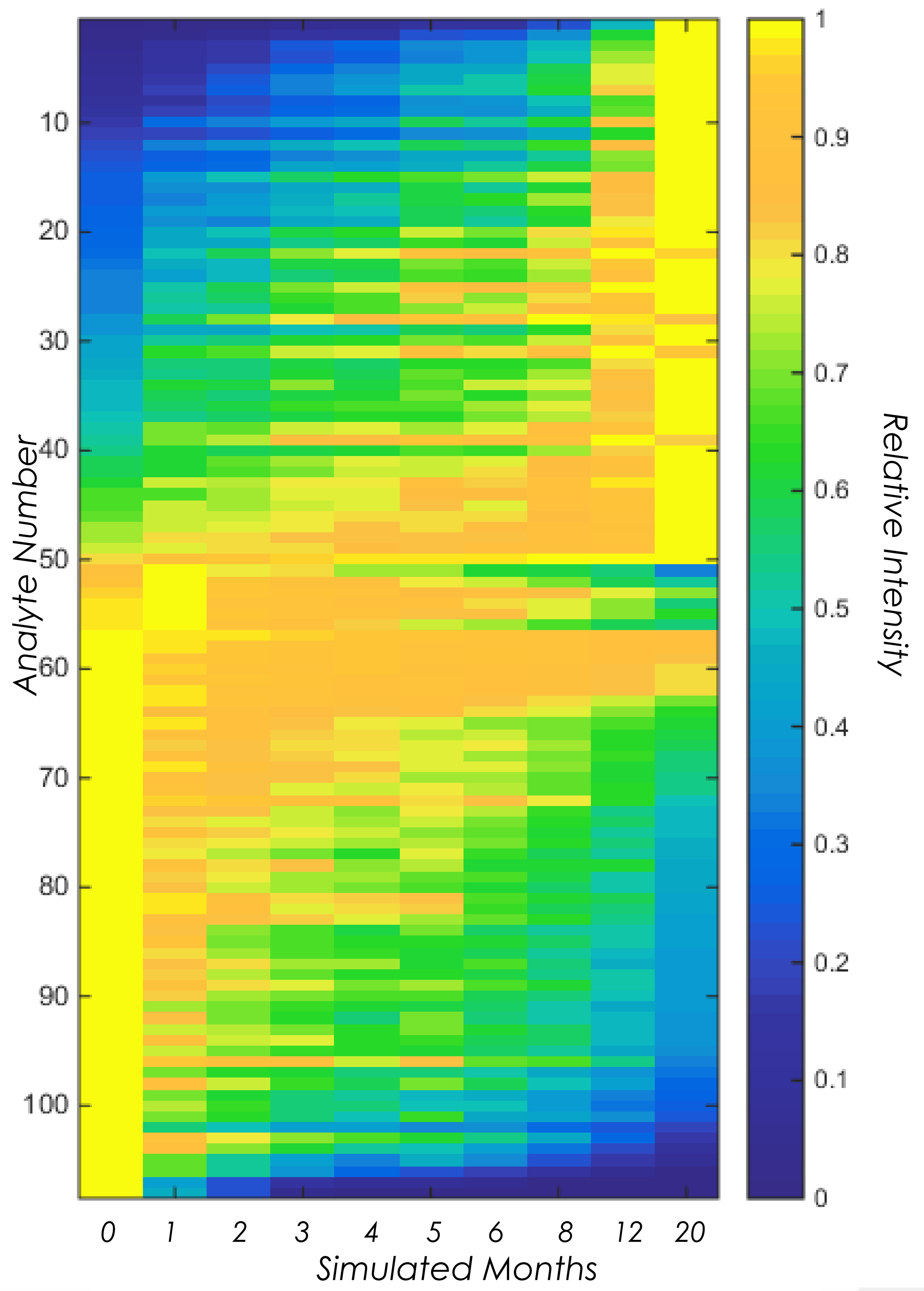

Figure 5. 108 Analytes that trend across the time course/aging process.

Representative Examples

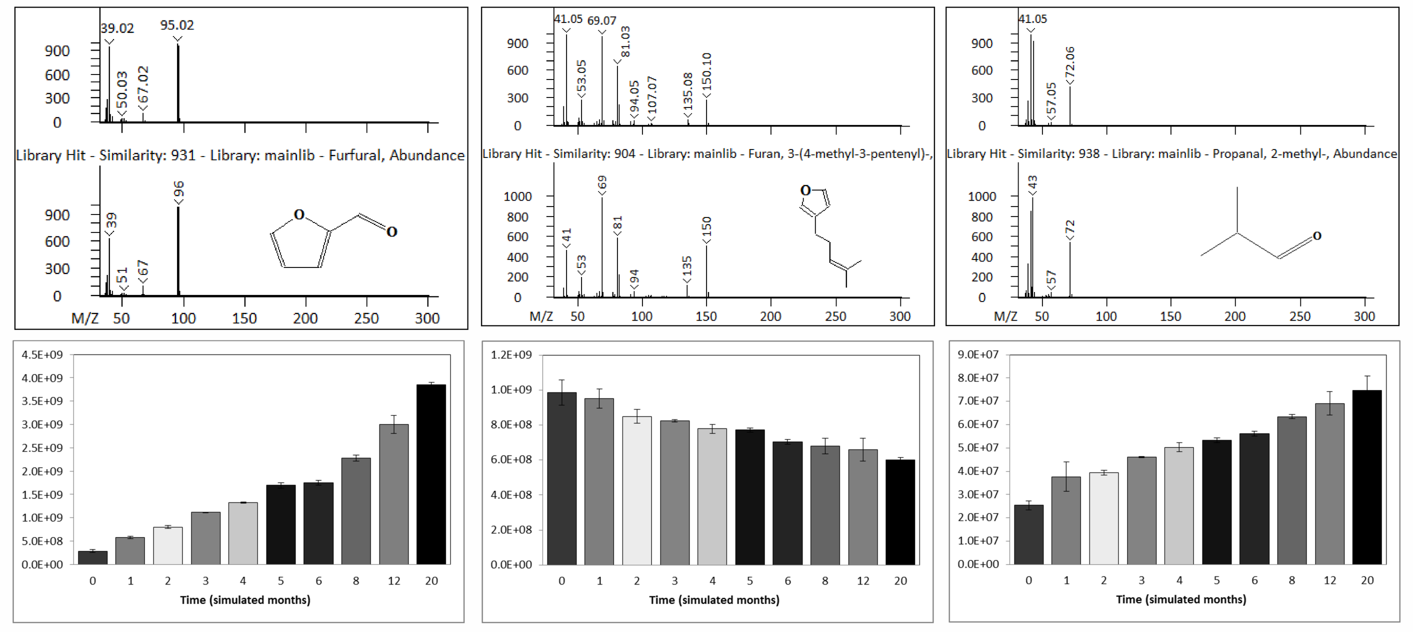

Figure 6. Representative analytes from heat map.

Table 2. Representative analytes and their odor properties

Peak#	Analyte	Similarity	Odor Notes
5	furfural	931	*sweet, woody, almond, fragrant baked bread*
6	2-methyl propanal	938	*pungent, cocoa, musty, green, malty, bready*
16	2-pentyl furan	866	*fruity, green, earthy, beany, vegetable*
19	2-n-butyl furan	837	*spicy, fruity, wine, sweet, mild*
23	α-terpinene	909	*woody, terpene, lemon, herbal, medicinal, citrus*
32	furan	906	*ethereal*
33	2 methyl furan	912	*chocolate, ethereal, acetone*
36	3-methyl ethyl ester butanoic acid	912	*fruity, sweet, apple, pineapple, tutti fruitti*
40	dimethyl sulfide	930	*sulfur, onion, sweet, corn, vegetable, cabbage, tomato, green, radish*
65	3-(4-methyl-3-pentenyl)- furan	904	*woody*
71	humulene	919	*woody*
98	caryophyllene	937	*sweet, woody, spice, clove, dry*
100	2 methyl octyl ester propanoic acid	914	*oily, green, waxy, soapy, clean, fruity, creamy*
104	α-pinene	888	*herbal, fresh, camphor, sweet, pine, earthy, woody*
108	caryophyllene oxide	868	*sweet, fresh, dry, woody, spicy*

Conclusions

This poster has demonstrated GC-TOFMS to monitor and track the aroma profile of aging beer. GC-TOFMS is a non-targeted analytical technique that provides chromatographic separation and full mass range mass spectral detection for the complete chromatographic separation. This comprehensive data can be mined for specific target analytes of interest, and also reviewed for inherent trends and differences in the data. Within a given compound class, various trends (increase, decrease, random variation, or no change) were observed for individual analytes, so non-targeted analysis tools were used to discover specific individual trending analytes. Peak area information for more than 300 analytes was compiled across the time course and 108 analytes that correlated with aging were determined. This analytical approach is useful for these types of discovery analyses.

Delivering the Right Results