A-102

Gas Chromatography with Time-of-Flight Mass Spectrometry for Aroma Profiling

Elizabeth M. Humston-Fulmer[1], Lucas R. Chadwick[2], and Joe Binkley[1]
LECO Corporation, St. Joseph, MI[1] and Bell's Brewery Inc., Galesburg, MI[2]

Introduction

Characterization of aroma compounds provides useful information in the food and beverage industry that can provide insight to quality control, process optimization, and brand awareness. Non-targeted methods that isolate and identify individual analyte components within the complex food matrix can provide a great deal of information. Here, methods were developed that utilize gas chromatography (GC) to separate individual analyte components from each other and time-of-flight mass spectrometry (TOFMS) for identification of the individual analytes. Aroma profile samples were prepared for analysis with headspace solid phase micro-extraction (HS-SPME) to collect and concentrate the volatile and semi-volatile compounds associated with a sample. These techniques were utilized to investigate both processing of raw materials and characterization of finished products. Changes in the aroma profile associated with the duration of boil time of hops were determined. The differences were readily apparent in the overall sample complexity and also clearly noted by changes to individual analyte concentrations. Characterization and comparison of final products was also accomplished with these analytical techniques. Principal component analysis (PCA) was used to investigate the similarities and differences of the sample groups with clear differences determined. These methods allowed for comparing sample types by their overall chromatographic features and by individual analyte differences in order to differentiate changes to a production process and to characterize beer samples.

Food Products and Methods

Hop Aroma Profiling

A hop extract was prepared from cascade leaf hops (Label Peelers, Kent, OH, USA) by adding 3 g of whole strobiles to 0.5 L of boiling water. Sample aliquots were removed from the boil after 5, 10, 20, 40, and 60 min of boiling and cooled. The overall volume of the boil was maintained at approximately 0.5 L with the addition of boiling water, as needed. Samples were prepared for HS-SPME analysis by adding 4.0 mL of each hop extract into 20 mL glass headspace vials. Sampling and instrument conditions are listed in Table 1.

Table 1. GC-TOFMS (Pegasus HT) Conditions for Hop Aroma Profiling

Extraction	Incubate for 10 min at 50°C then extract 30 min at 50°C with 50/30 µm DVB/CAR/PDMS fiber (Supelco, Bellefonte, PA)
Injection	Analytes were desorbed from the fiber and injected for analysis by exposing the fiber in a 250°C GC-inlet for 2 min.
Carrier Gas	He (ultra high purity) @ 1.0 ml/min
Column	Rxi-5Sil MS, 30 m x 0.25 mm x 0.25 µm film coating (Restek, Bellefonte, PA)
Temp Program	4 min at 35°C, ramped 10°C/min to 250°C and held 4 min
TOFMS Conditions	30-400 m/z at 20 spectra/s with source temp of 250°C

Beer Aroma Profiling

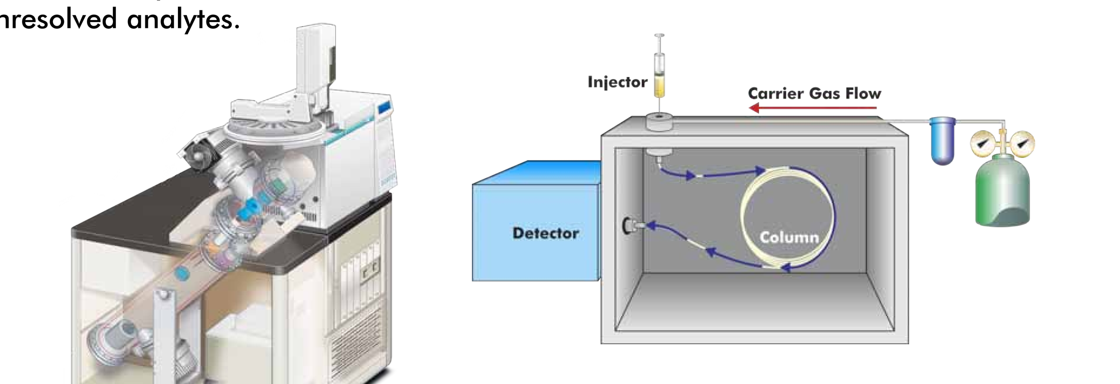
A variety of commercially available craft beer styles, including stouts and India pale ales (IPAs), were analyzed and compared with GC-TOFMS. Aliquots of 4.0 mL were sampled by HS-SPME. Sampling and instrument conditions are listed in Table 2.

Table 2. GC-TOFMS (Pegasus HT) Conditions for Beer Aroma Profiling

Extraction	Incubate for 10 min at 50°C then extract 10 min at 50°C with 50/30 µm DVB/CAR/PDMS fiber (Supelco)
Injection	Analytes were desorbed from the fiber and injected for analysis by exposing the fiber in a 250°C GC-inlet for 2 min.
Carrier Gas	He (ultra high purity) @ 1.0 ml/min
Column	Rxi-5Sil MS, 30 m x 0.25 mm x 0.25 µm film coating (Restek)
Temp Program	2 min at 40°C, ramped 10°C/min to 250°C and held 2 min
TOFMS Conditions	33-510 m/z at 15 spectra/s with source temp of 250°C

GC-TOFMS

GC-TOFMS isolates individual analytes within a complex sample for identification, quantification, and non-targeted characterization. Individual analytes are separated chromatographically and subsequently measured by TOFMS. With complex samples, some coelution is common, and multiple analytes may reach the TOFMS detector simultaneously. In these cases, mathematical deconvolution can often isolate the unresolved analytes.



Pegasus HT Instrument Schematic

Identify Individual Analytes

GC-TOFMS with mathematical deconvolution provided the isolation and identification of hundreds of analytes from a complex hop aroma sample. Representative analyte examples and their associated aroma properties are shown.

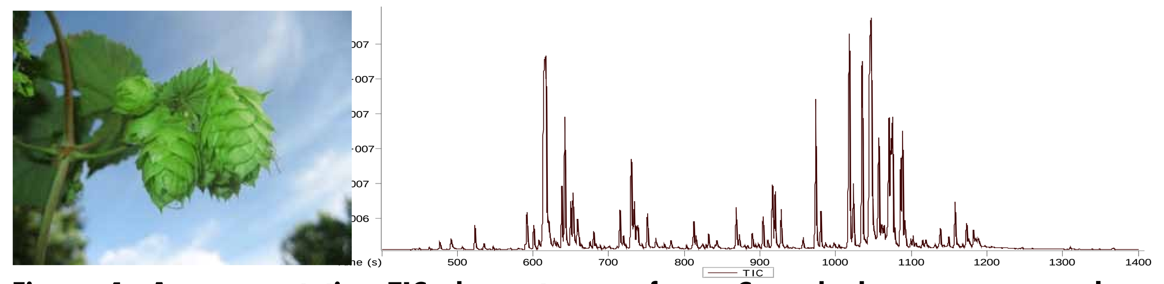


Figure 1. A representative TIC chromatogram for a Cascade hop aroma sample separates individual components within the complex sample.

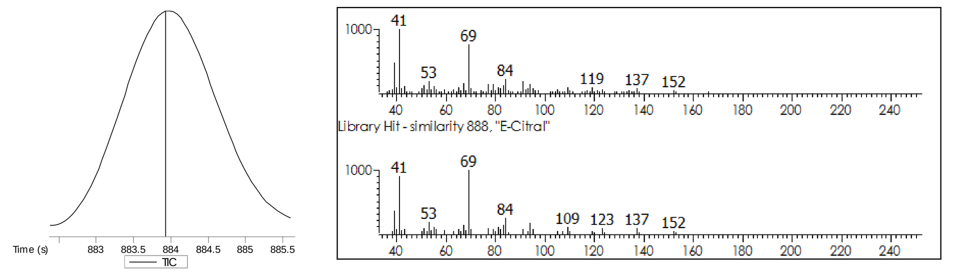


Figure 2. A chromatographically isolated analyte from the hop aroma sample was rapidly identified through library searching of the TOFMS data. E-citral is a compound with lemony/citrusy odor properties.

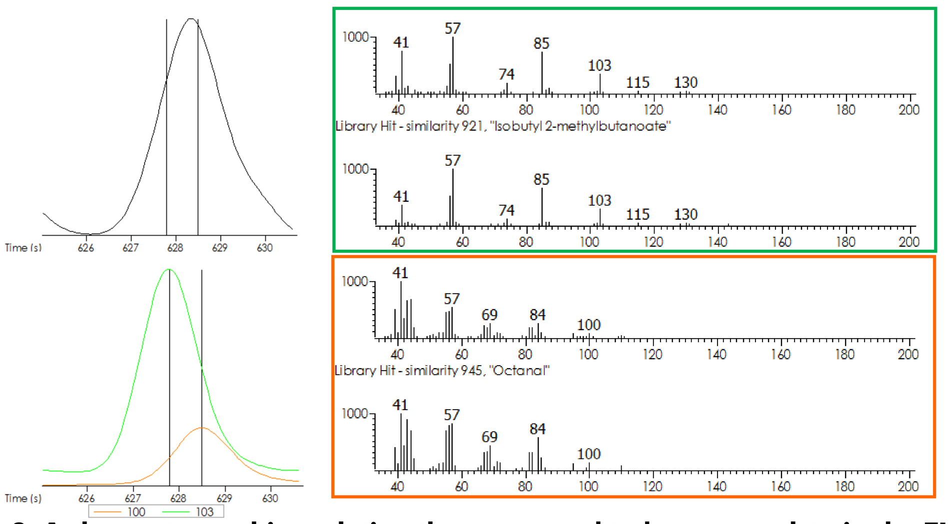


Figure 3. A chromatographic coelution that appeared to be one analyte in the TIC view was mathematically deconvoluted as two analytes, visualized through unique ions (m/z 100 and 103). These analytes were identified as isobutyl 2-methylbutanoate and octanal with fruity/citrusy flavors and citrus/orange flavors, respectively.

Hop Aroma Profile

These methods and instrumentation provided good characterization of the complex hop aroma samples with the ability to isolate and identify many analyte types, including: esters, alkanes, aldehydes, carboxylic acids, terpenes, etc. This allowed for investigating changes related to the length of boil time for hops, which is known to have a profound impact on the associated aroma and flavor properties.

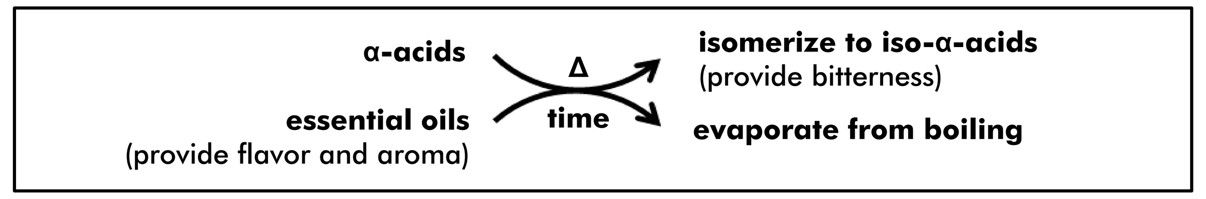


Figure 4. Hop flavor and aroma primarily comes from α-acids and essential oils. The α-acids require heat to isomerize to iso-α-acids which simultaneously leads to the loss of other flavor and aroma analytes.

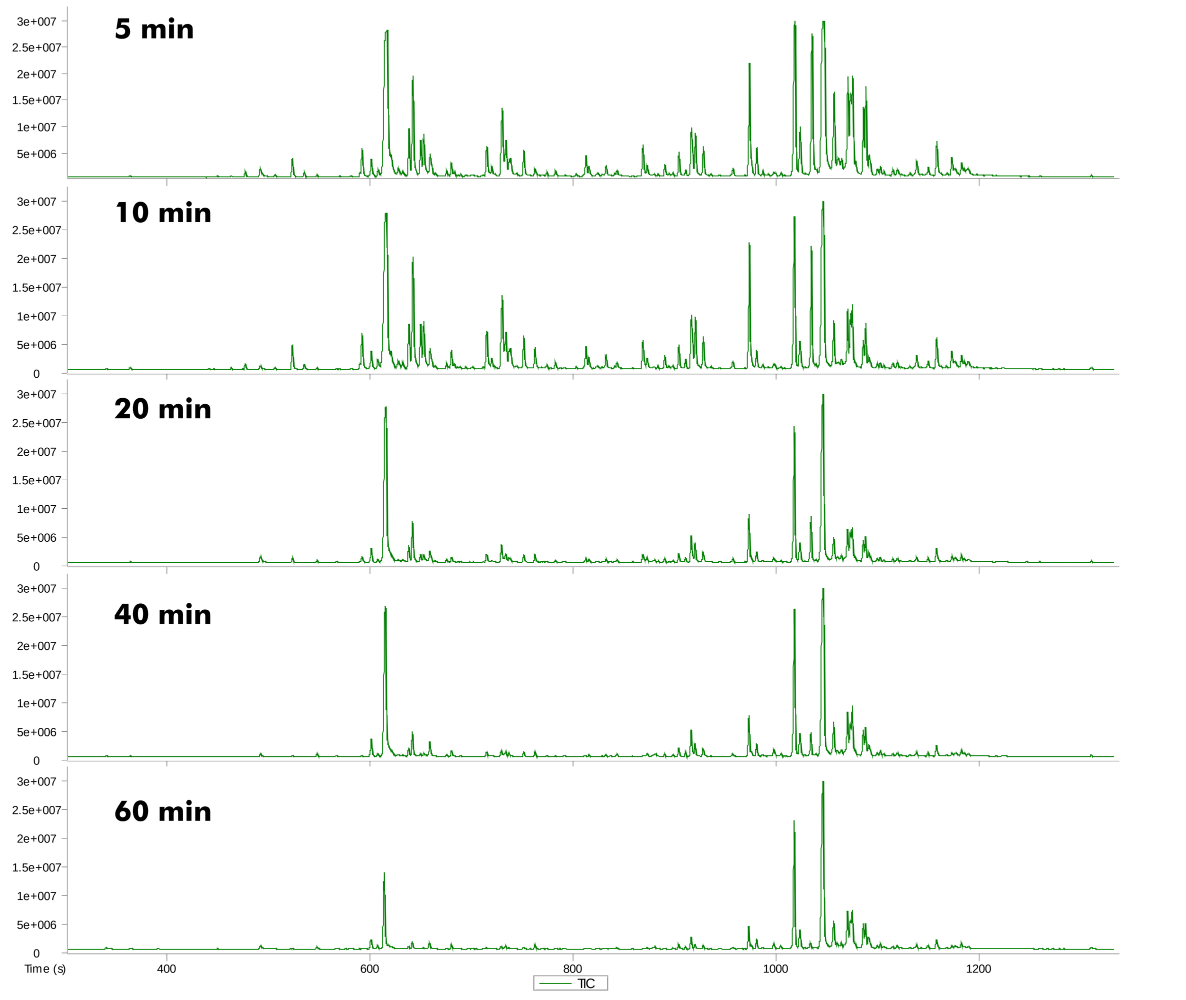


Figure 5. GC-TOFMS TIC chromatograms for the 5, 10, 20, 40, and 60 min boil times. Clear differences were observed. Samples exposed to longer time periods of boiling, had fewer and less intense volatile and semi-volatile analyte peaks in the chromatograms.

LECO's ChromaTOF software was utilized to rapidly determine analyte identification (tentatively through library matching) and compare 18 hop aroma compounds known to contribute to flavor and aroma. Consistently across these analytes, a loss of intensity was observed after 10 min of boiling. On average, the levels at 20 min are less than 40% of those observed after 5 min of boiling.

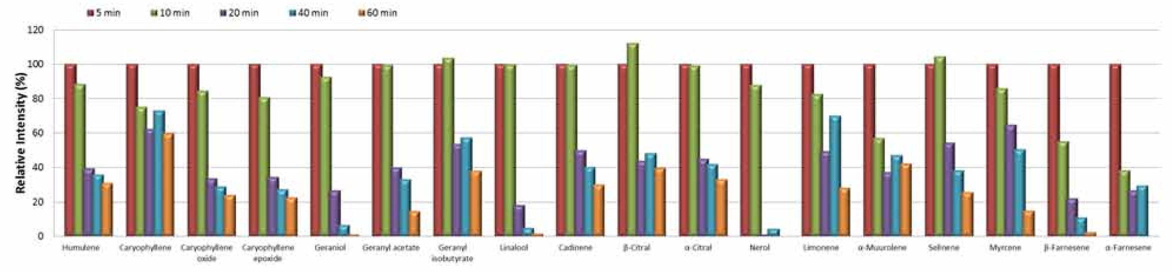


Figure 6. Representative analytes are shown with relative intensity as a function of boil time. The 5 min boil time is set to 100%.

Beer Aroma Profiling

These analysis tools also provide differential analysis capabilities to gain insight to finished products. This type of aroma profiling information can be useful for quality control purposes, connecting sensory observations to chemical properties, brand awareness, screening for off-flavors or adulterants, and for product development to adjust or mimic particular flavors. A selection of commercially available beer samples were analyzed with these methods and characterized.

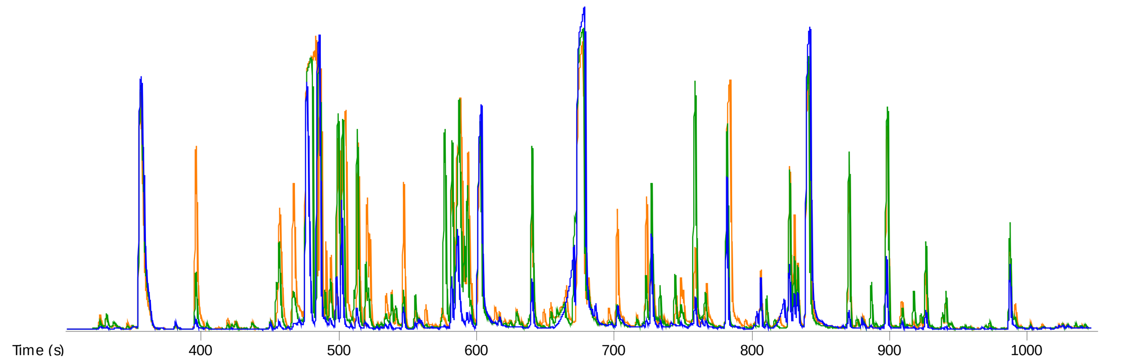


Figure 7. Three beer types, pale ale (blue), IPA (orange), and double IPA (green) were compared. Representative TIC chromatograms are shown overlaid.

The chromatographic traces were used for fingerprinting with Principle Component Analysis (PCA) to classify the samples. The samples clearly clustered in the scores plot and many analyte differences were determined. A collection of representative highly loaded variables are shown.

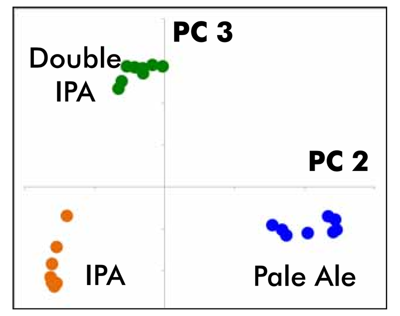
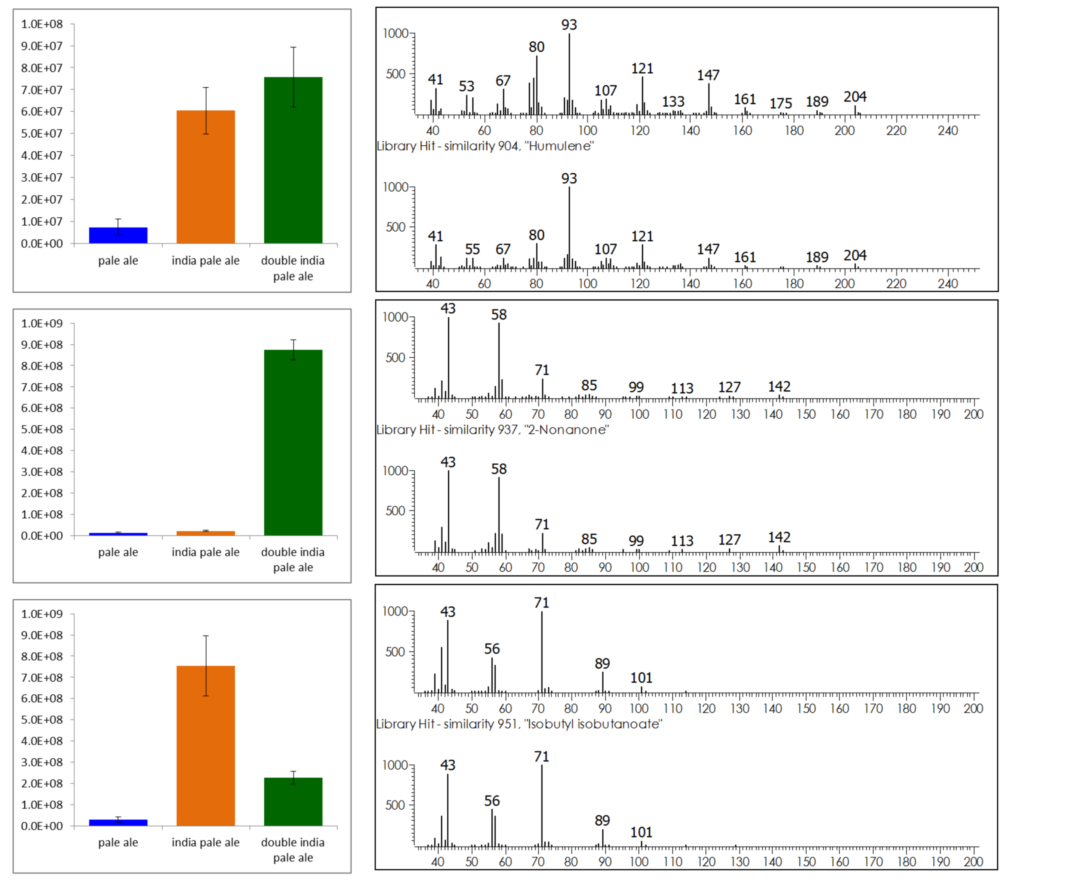


Figure 8. PCA scores plot. The chromatographic traces were submit as a fingerprints to PCA, resulting in distinct clusters of the samples by product.



Analyte	Similarity	Aroma and Flavor Notes
humulene	904	Present in hop oil, wood odor
2-nonanone	937	Present in beer, fruity odor
isobutyl isobutanoate	951	Present in hop oil, fruity/green apple odor

Figure 9. A collection of representative analytes that differed between brands. These analyte differences are likely due to both the hop level and hop variety used for each brand. Many other analyte differences were observed.

Beer Aroma Profiling

A variety of flavored beers were analyzed to compare and characterize the products. A fruit-flavored and coffee-flavored stout were compared to a stout. Specific analyte differences between the brands that contribute to the fruit and coffee flavor notes were determined.

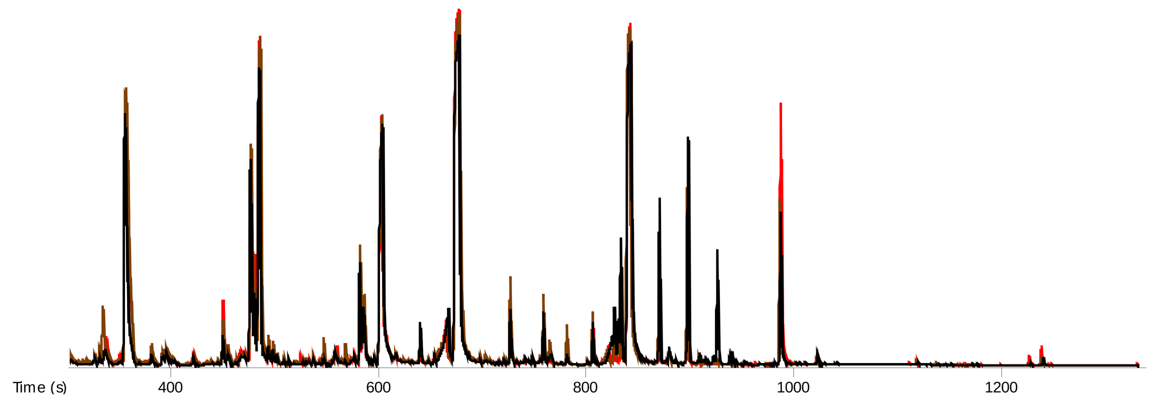
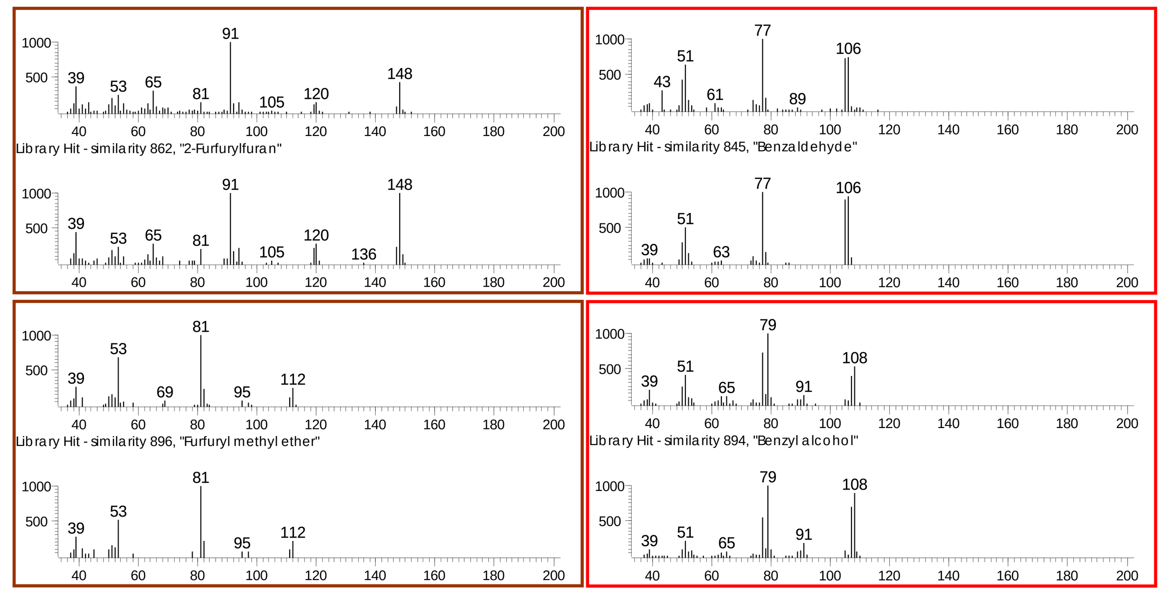


Figure 10. Three beer brands, a stout (black), coffee stout (brown), and cherry stout (red) were analyzed with these tools and compared.



Analyte	Similarity	Elevated in	Aroma and Flavor Notes
2-furfuryl furan	862	Coffee	Present in coffee, rich and roasted odors
furfuryl methyl ether	896	Coffee	Present in coffee, roasted coffee odor
benzaldehyde	845	Cherry	Fruity, nutty, and cherry odor
benzyl alcohol	894	Cherry	Fruity odor

Figure 11. A collection of representative analytes determined to be unique to the flavored brands are shown. These analytes contribute to the differences in sensory observations. Many other analyte differences were observed.

Conclusions

This poster has demonstrated GC-TOFMS instrumentation applied to characterize beer-related samples. The ability to isolate and identify individual analytes through chromatographic resolution, mathematical deconvolution, and mass spectral searching to library standards provides good insight to various stages of production. A process monitoring application was highlighted in which aroma changes related to the length of boil time for hops were identified. This type of information could be used to optimize hop addition times in the process to achieve desired sensory characteristics. Differential analysis of various brands was also demonstrated with comparisons of products with different levels of hop characteristics and products of various flavored stouts. The chromatographic traces were used as chemical fingerprints for PCA to classify and cluster the samples. Specific analyte differences were determined with representative examples shown. This type of aroma profiling information can be useful for quality control purposes, connecting sensory observations to chemical properties, and for product development to adjust or mimic particular flavors. These methods allowed for characterizing sample types by their overall chromatographic features and by individual analyte differences. These tools are very powerful and have broad applicability in the brewing industry.