Analysis of Hops Aroma Profiles as a Function of Boil Time by HS-SPME Using GC-TOFMS and GCxGC-TOFMS

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Background

Monitoring flavors associated with food products is important in the food and beverage industry. Ensuring consistency in flavors can provide a measure of quality control, and changes in the flavor and aroma notes indicative of processing methods or quality can be determined. This information can be used to drive modifications in optimizing a food item or a production method. Flavor profiles are comprised of the volatile and semi-volatile compounds that contribute to the characteristic aroma of a food item and generally consist of a large number of compounds that span a range of concentrations. In this poster, a method is developed to characterize aroma and flavor compounds associated with hops, *Humulus lupulus*, throughout the boiling stage of the beer brewing process. The timing of the hop addition during beer brewing can have a large impact on the eventual aroma and flavor profile of the final product. The boil process was simulated and headspace solid-phase micro-extraction (HS-SPME) was used to sample the volatile and semi-volatile aroma and flavor compounds in the headspace of a boiled hop flower extract. Both one- and two-dimensional gas chromatography (GC and GCxGC) with Time-of-Flight Mass Spectrometry (TOFMS) were subsequently used to separate, quantify, and identify these compounds. Target analytes were monitored throughout the boil and quantified in order to determine aroma and flavor changes as a function of boil time.

Flavors of Hops

Figure 1. Photograph of hops flowers.

Hops, shown in Figure 1, are one of the primary ingredients used in brewing and serve as both a natural preservative and as a flavoring agent. The leafy green flowers are responsible for the characteristic bitterness in beer, but also impart other flavors such as floral, tangy, piney, or citrusy notes. Hop variety is one factor that impacts the flavor of beer, as different strains lead to different flavors and aromas. Another important aspect is the timing of the hop addition during brewing.

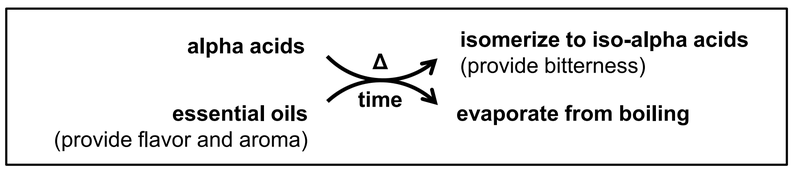

Figure 2. Flavor and aromas primarily come from alpha acids and essential oils. Alpha acids in hops require extended exposure to heat to isomerize into iso-alpha acids, but the thermal energy from the extended boil simultaneously leads to a loss of the essential oils responsible for other flavors and aromas. Thus, hops are generally added multiple times during brewing; earlier to provide bitterness and later for aroma and flavor.

Methods

Samples: Cascade Leaf Hops were purchased from Label Peelers (Kent, OH, USA). A hop extract was prepared by adding 3 g of hop flowers to 0.5 L of boiling water to mimic the boil process. This ratio of hops-to-water is roughly equivalent to 4 oz. of hops in a 5 gallon batch. Sample aliquots of 20 mL were removed from the boil at 5, 10, 20, 40, and 60 minutes of boil time and cooled. The overall volume of the boil was maintained at approximately 0.5 L with the addition of boiling water as needed.

SPME Conditions: 4.0 mL of each hop extract were transferred into 20 mL glass headspace vials then sealed with septum caps. The extraction was automated using a GERSTEL MPS2 Auto Sampler through LECO's ChromaTOF® software. Samples were incubated at 50°C for 10 min immediately prior to extraction. A 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/Carb/PDMS) SPME fiber (Supelco, Bellefonte, PA, USA) was exposed to the sample headspace for 30 minutes at 50°C. Analytes were desorbed from the fiber and injected for analysis by exposing the fiber in a 250°C GC-inlet for 2 minutes.

Instrumental Conditions: GC analyses were performed with LECO's Pegasus HT consisting of an Agilent 6890 GC equipped and LECO Pegasus TOFMS. GCxGC analyses were performed with LECO's Pegasus 4D, consisting of an Agilent 7890 GC equipped with LECO's dual stage quad jet thermal modulator, secondary oven, and Pegasus TOFMS.

Table 1. Instrument Method Parameters

GC-TOFMS (Pegasus HT) Conditions	
Carrier Gas	He @ 1.0 ml/min
Column	Rxi-5Sil MS, 30 m x 0.25 mm x 0.25 µm (Restek, Bellefonte, PA)
Temp Program	4 min at 35°C, ramped 10°C/min to 250°C and held 4 min
Mass Range	30-400 m/z
Acquisition Rate	20 spectra/s
Source Temp	250°C

GCxGC-TOFMS (Pegasus 4D) Conditions	
Carrier Gas	He @ 1.0 ml/min
Column One	Rxi-5Sil MS, 30 m x 0.25 mm x 0.25 µm (Restek, Bellefonte, PA)
Column Two	Stabilwax, 1.5 m x 0.25 mm x 0.25 µm (Restek, Bellefonte, PA)
Temp Program	4 min at 35°C, ramped 5°C/min to 250°C, held 4 min; Secondary oven maintained +10°C relative to primary
Modulation	6 s with temperature maintained +20°C relative to 2nd oven
Mass Range	30-400 m/z
Acquisition Rate	100 spectra/s
Source Temp	250°C

Mass Spectral Deconvolution

Isolating individual analytes within a complex matrix can yield useful insight to the sample. Chromatographic resolution is the first level of isolating individual analytes, but complex samples generally still contain some unresolved regions. Chromatographically overlapped analytes can often be separated based on differences in their mass spectral patterns, with Deconvolution algorithms such as ChromaTOF's True Signal Deconvolution®.

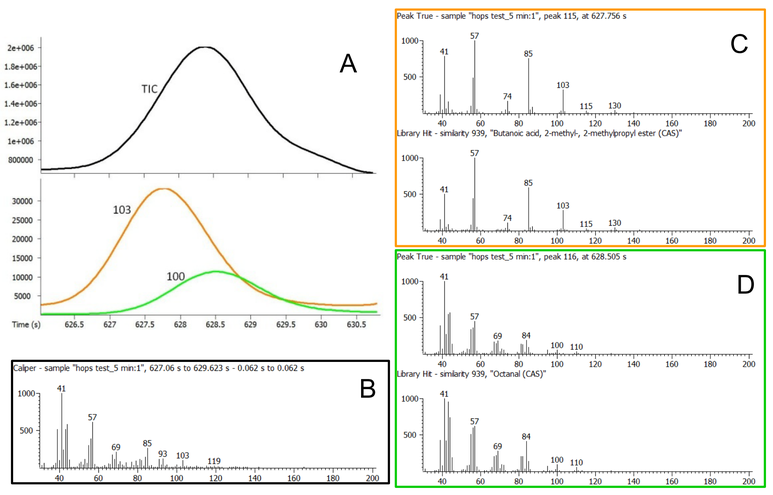

Figure 3. ChromaTOF's True Signal Deconvolution resolved chromatographically overlapped analytes. What appeared as one analyte in the TIC view (A) was deconvoluted to two unique analytes, visualized through unique ions. The mass spectral data across the width of what appeared to be a single peak (B) was isolated into (C) 2-methyl 2-methylpropyl ester butanoic and (D) octanal.

Deconvolution of the mass spectral data provides quantification and identification of each analyte through integration of unique m/z and library matching of the pure spectra, respectively. Prior to deconvolution, the mass spectral data in Figure 3B did not match well to any library spectra. Library searching of the deconvoluted mass spectral data, however, resulted in matches to 2-methyl 2-methylpropyl ester butanoic acid, with fruity and citrusy flavors, and octanal, with citrus and orange flavors shown in Figure 3C and 3D, respectively.

GCxGC Benefits

Another way to separate analytes which were not chromatographically resolved in a 1D separation, especially in instances where mass spectral deconvolution is unable to separate coeluting analytes, is to add a second separation dimension, as with GCxGC. GCxGC provides two main benefits for the analysis of complex samples. First, an improved peak capacity with two complementary separations. Second, the cryogenic focusing of thermal modulation provides low-level detection capabilities. Examples of these benefits are shown in Figure 4, the region of the chromatogram between 2-decanone and decanal (815 to 829 and 1200 to 1224 in the 1D and 2D data, respectively).

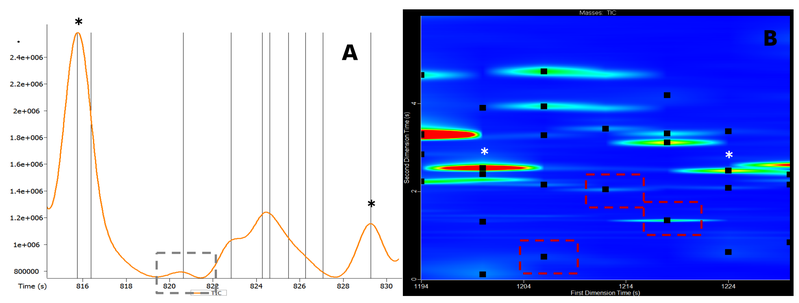

Figure 4. (A) GC-TOFMS and (B) GCxGC-TOFMS chromatograms showing 2-decanone through decanal, both indicated with asterisks. Peaks are indicated in the 1D separation by vertical lines and by black dots in the 2D separation

In the 1D separation, there are 10 peaks between and including these two analytes, and in the 2D separation there are 20 peaks. The increase in detected peaks can be attributed to both lower-level detection and the ability to separate previously coeluting analytes. In the GC-TOFMS data shown in Figure 4A, the small peak at 820.6 s, enclosed in the dashed gray box, is identified as 2-hydroxy-methyl ester benzoic acid with a match value of 638. The library and true peak spectra are shown in Figure 5A. Multiple interfering m/z (55, 71, 81, and 96) all lead to the fairly low match value. In the GCxGC separation, this peak is chromatographically separated into 3 separate peaks, shown enclosed in red dashed boxes in Figure 4B. The spectra for these three analytes are shown in Figure 5B-5D and roughly combine to the single peak spectra from the GC-TOFMS data. This additional peak capacity leads to both a higher match value for 2-hydroxy-methyl ester benzoic acid (916 instead of 638) and the ability to measure additional analytes.

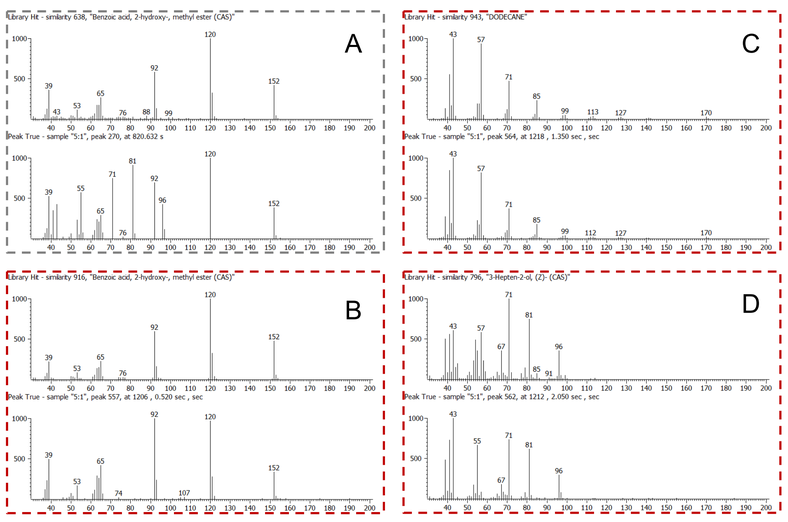

Figure 5. MS data for the analytes highlighted in Figure 4. GCxGC chromatographically resolves coeluting GC peaks.

Time Dependencies for Aroma and Flavor

These methods and instrumentation provided good characterization of the complex hop aroma samples and allowed for monitoring time dependencies of specific flavor and aroma compounds. Clear differences between the TIC chromatograms of both the GC-TOFMS and GCxGC-TOFMS analyses for the 5 and 60 minute boil times can be observed in Figure 6. Samples exposed to longer time periods of boiling had fewer volatile and semi-volatile analyte peaks in their chromatograms. A total of 607 and 373 peaks with S/N >200 were detected in the hops sample boiled for 5 minutes and 60 minutes, respectively. With the benefits of GCxGC, the total number detected peaks increased to 1057 and 500 peaks with S/N >200 in the 5 minute and 60 minute samples, respectively.

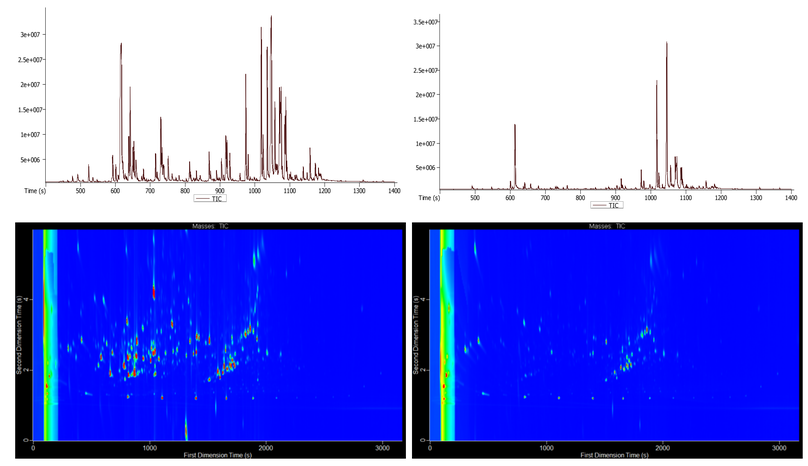

Figure 6. GC-TOFMS (top) and GCxGC-TOFMS (bottom) TIC chromatograms for the 5 minute boil time (left) and 60 minute boil time (right).

The information for all peaks within the data is automatically compiled into Peak Tables for review. This allows for gleaning information on both targeted and untargeted analytes. Reverse Library Searches can also facilitate locating specific target analytes by matching library spectra to the data to locate the analyte peak with the best match. These tools were utilized to locate and tentatively identify the 18 target hop aroma compounds listed in Table 2 in the GC-TOFMS data.

Table 2. Target Aroma and Flavor Compounds from GC-TOFMS Data

HOP AROMA COMPOUNDS	tR	m/z	MV
Humulene and Caryophyllene Oxidation Products			
Humulene	1149.7	80	827
Caryophyllene	1047.82	80	906
Caryophyllene oxide	1138.72	109	854
Caryophyllene epoxide	1158.19	109	822
Floral/Estery Compounds			
Geraniol	868.855	93	897
Geranyl acetate	974.086	121	900
Geranyl isobutyrate	957.612	45	901
Linalool	730.327	93	819
Citrus/Piney Compounds			
D-Cadinene	1176.81	161	868
Citral (Z)	858.821	119	861
Citral (E)	883.931	84	885
Nerol	847.139	136	890
Limonene	659.091	68	933
a-Muurolene	1102.73	105	892
Selinene	1190.19	189	859
Other			
Myrcene	617.108	53	805
Farnesene (b)	1035.84	69	905
Farnesene (a)	1061.7	107	828

ChromaTOF software contains a Reference feature that allows for rapid comparisons of samples to each other, including relative concentrations of analytes to each other across a set of samples. This feature was used to quantify the target analytes in all of the time course samples for time course comparison purposes. Quantitative information is compiled graphically in Figure 7. In this application of the Reference feature, the 5 minute boil time was set as the Reference and the 18 target analytes were quantified relative to the 5 minute sample in all other samples.

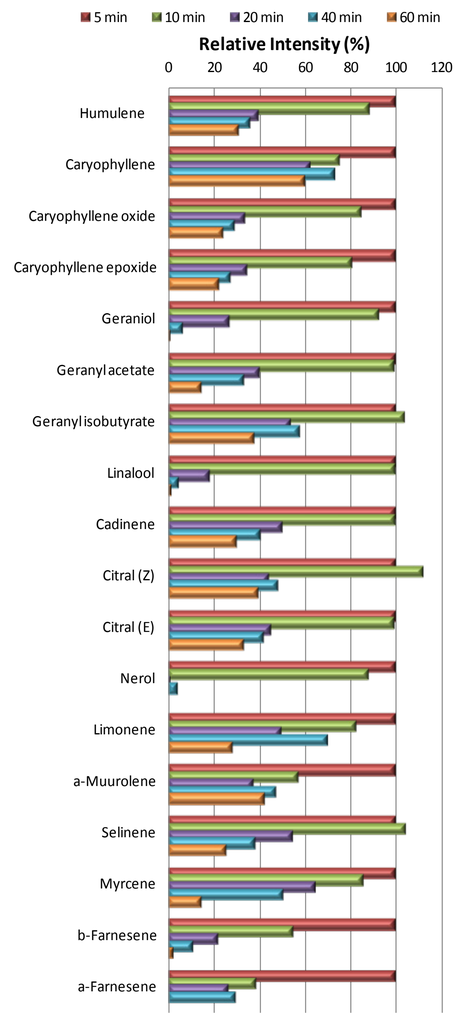

Figure 7. Target analytes (tentatively identified by mass spectral matching) are shown with relative intensity as a function of boil time. The 5 minute boil time is set to 100%.

Consistently across all 18 target aroma compounds, a loss of intensity is observed after the 10 minute boil time. On average, the levels at 20 min are less than 40% of those observed after 5 minutes of boiling. Many analytes are not detected or are detected at only low levels by the 60 minute boil time. These data are consistent with the understanding that hops should be added late in the boil to optimize their aroma and flavor contributions. The detection of the iso-alpha acids, responsible for bitterness, is not compatible with these sampling conditions, so their presumed increase is not apparent in these data.

Conclusions

The experiments described in this poster demonstrate a food, flavor, and fragrance analysis for the characterization of hops aroma profile. HS-SPME was used to pre-concentrate volatile and semi-volatile compounds in a hops extract. LECO's Pegasus HT GC-TOFMS efficiently separated, quantified, and identified analytes within the complex sample matrix. Full mass range acquisition allowed for identification of both target and non-target compounds through mass spectral matching to library spectra. The target aroma compounds were quantified across all boil time samples to determine temporal dependencies, providing information on the relative contribution of aromatic and flavor notes based on the timing of the hop addition. LECO's Pegasus 4D GCxGC-TOFMS also efficiently separated individual analytes for identification and quantification, offering additional peak capacity and lower level detection in order to increase the number of measured analytes.