

# Characterization of Food Products by GC×GC-TOFMS and GC-High Resolution TOFMS: A Food “omics” Approach

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## Introduction

Gas chromatography (GC) paired with mass spectrometry (MS) is an effective tool for characterizing and distinguishing food products. Individual analytes can be isolated from complex food matrices with GC and identified with MS. This type of information provides food “omics” insight at various stages throughout production, including differentiation of raw materials, process changes, and finished products. As sample complexity increases, additional resolution can be gained with mathematical deconvolution of TOFMS data, two-dimensional GC (GC×GC), and/or with high-resolution MS (HR-TOFMS) to help isolate even more individual analytes. These analytical tools allowed for comparing food products by their chromatographic fingerprints, characterizing samples with the identification of individual analyte differences, and differentiating samples with PCA. These capabilities have implications in quality control, process optimization, and detection of food fraud, among others.

## Food Products and Methods

GC with TOFMS was utilized to investigate a variety of food items including hops, beer, and edible oils. For each food type, HS-SPME sampling (50/30 μm DVB/CAR/PDMS, Supelco, Bellefonte, PA) prepared the sample for analysis with LECO’s Pegasus® HT GC-TOFMS, Pegasus 4D GC×GC-TOFMS, and/or Pegasus GC-HRT. Specific sample details are provided in Tables 1-3.

**Table 1. Conditions for Hop Aroma Profiling**

<b>Samples</b>	3 g of whole cascade leaf hops (Label Peellers, Kent, OH) were added to 0.5 L of boiling water. Hop extract aliquots were taken after 5, 10, 20, 40, and 60 min of boiling.
<b>Pegasus HT GC-TOFMS</b>	
<b>GC Conditions</b>	4 min at 35°C, ramped 10°C/min to 250°C and held 4 min on a 30 m x 0.25 mm x 0.25 μm Rxi-5Sil MS (Restek, Bellefonte, PA)
<b>TOFMS Conditions</b>	30-400 m/z at 20 spectra/s with source temp of 250°C

**Table 2. Conditions for Beer Aroma Profiling**

<b>Samples</b>	A variety of commercially available craft beer styles including stouts and India pale ales (IPAs) were analyzed.
<b>Pegasus HT GC-TOFMS</b>	
<b>GC Conditions</b>	2 min at 40°C, ramped 10°C/min to 250°C and held 2 min on a 30 m x 0.25 mm x 0.25 μm Rxi-5Sil MS (Restek)
<b>TOFMS Conditions</b>	33-510 m/z at 15 spectra/s with source temp of 250°C

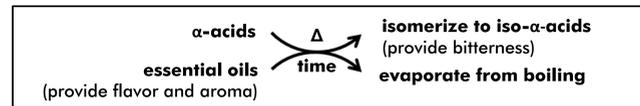
**Table 3. Conditions for Edible Oil Characterization**

<b>Samples</b>	Three extra virgin olive oil varieties and one each of olive, vegetable, peanut, and grapeseed oil were analyzed. Oil mixtures were also prepared to simulate food fraud.
<b>Pegasus 4D GC×GC-TOFMS</b>	
<b>GC Conditions</b>	2 min at 40°C, ramped 12°C/min to 250°C, and held 3 min on a 30 m x 0.25 mm x 0.25 μm Rxi-5Sil MS paired with a 1.3 m x 0.18 mm x 0.18 μm Rxi-17Sil MS (Restek). Modulation every 2 s with modulator temp maintained +15°C relative to secondary oven and secondary oven maintained +5°C relative to primary
<b>TOFMS Conditions</b>	33-400 m/z at 200 spectra/s with source temp of 250°C
<b>Pegasus GC-HRT (GC-HR-TOFMS)</b>	
<b>GC Conditions</b>	2 min at 40°C, ramped 12°C/min to 250°C, held 3 min on a 30 m x 0.25 mm x 0.25 μm Rxi-5Sil MS (Restek)
<b>TOFMS Conditions</b>	33-400 m/z at 6 spectra/s with source temp of 250°C High Resolution Mode at 25,000

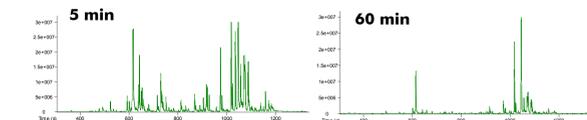
Data analyses were accomplished with ChromaTOF® software for peak finding, mathematical deconvolution, and identification; Excel for data compilation; and XLStat for chemometric analysis. TIC traces were utilized for fingerprinting and individual analytes were identified through library matching of nominal mass spectra and accurate mass information in high resolution data, when available.

## Hop Aroma Profile

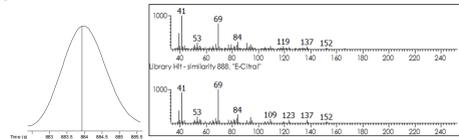
GC-TOFMS with mathematical deconvolution allowed for investigating changes related to the length of boil time for hops, known to have a profound impact on the associated aroma and flavor properties. These methods provided good characterization of complex hop aroma samples with the isolation and identification of many analyte types, including: esters, alkanes, aldehydes, carboxylic acids, terpenes, etc. These tools provided information on individual analytes and time course trends which could aid in process development and optimization to achieve particular sensory traits.



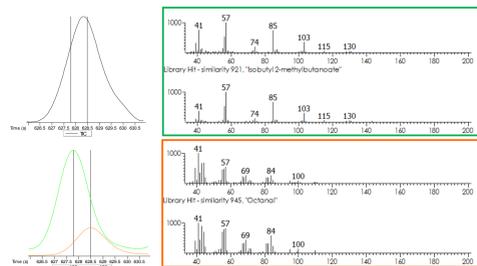
**Figure 1.** Much of hop flavor and aroma comes from iso-α-acids and essential oils. Heat is required to isomerize α-acids to iso-α-acids, which simultaneously leads to the loss of the essential oils responsible for other flavor and aroma analytes.



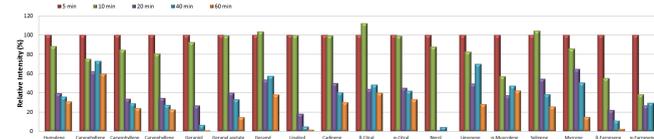
**Figure 2.** GC-TOFMS TIC chromatograms for the 5 and 60 min boil times. Samples exposed to longer boil times had fewer and less intense volatile and semi-volatile analyte peaks in the chromatograms.



**Figure 3.** Individual analytes were isolated chromatographically and rapidly identified through library searching. For example, E-citral with lemony/citrusy odor properties was isolated and identified.



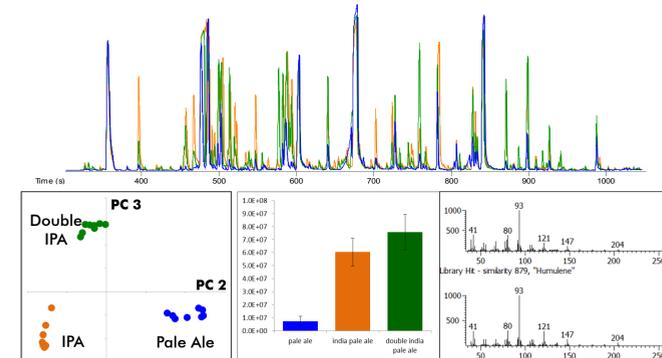
**Figure 4.** Chromatographic coelutions were often resolved with mathematical deconvolution. Two coeluting analytes (visualized through unique ions m/z 100 and 103) were isolated and their pure spectra were identified as isobutyl 2-methylbutanoate and octanal with fruity/citrusy and citrus/orange flavors, respectively.



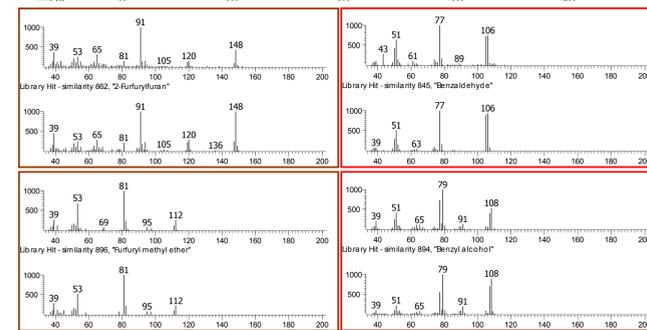
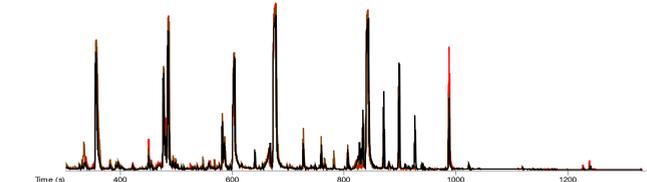
**Figure 5.** Eighteen representative analytes are shown with relative intensity as a function of boil time. Consistently for these analytes, a loss of intensity was observed after 10 min of boiling. The 5 min boil time was set to 100% and, on average, the levels at 20 min were less than 40% of those observed after 5 min of boiling.

## Beer Aroma Profile

These analytical tools also provide differential analysis capabilities to gain insight to a finished product. This type of aroma profiling 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 and characterized.



**Figure 6.** Three beer varieties (pale ale, IPA, and double IPA) were compared. The TIC traces were submitted as fingerprints to PCA to classify the samples. Humulene, an analyte naturally present in hops, was one of the highly loaded variables and correlated to increasing hop characteristic in the samples. Many other analyte differences were observed and identified.

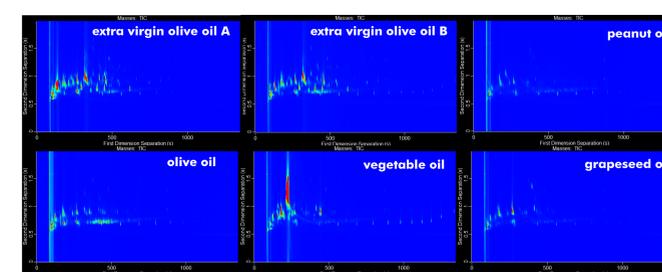


Analyte	Similarity	Elevated in	Aroma and Flavor Notes
2-furfuryl furan	862	Coffee	Present in coffee, rich and roasted odors
furfuryl methyl ether	896	Coffee	Present in coffee, roasted coffee odor
benzaldehyde	845	Cherry	Fruity, nutty, and cherry odor
benzyl alcohol	894	Cherry	Fruity odor

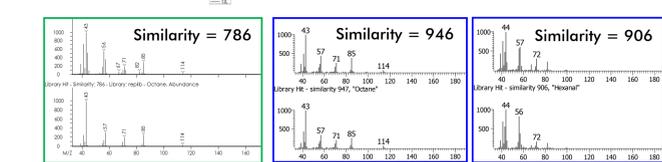
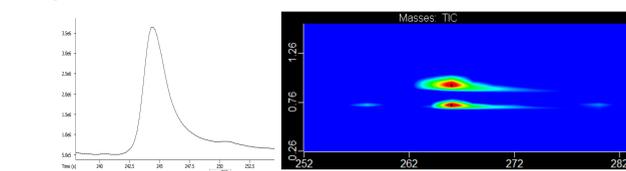
**Figure 7.** Three beer varieties—a stout (black), coffee stout (brown), and cherry stout (red)—were analyzed with these tools and compared. A collection of representative analytes determined to be unique to the flavored stouts are shown. These analytes contribute to the differences in sensory observations. Many other analyte differences were observed.

## Edible Oil Characterization

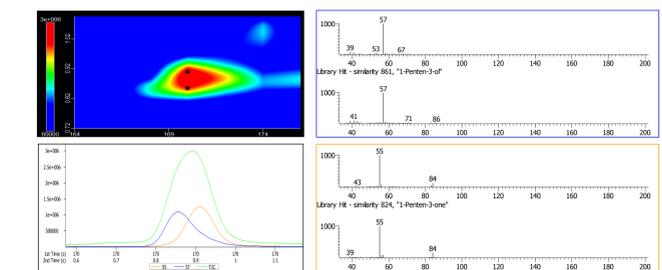
Adulteration of extra virgin olive oil by blending in cheaper oil varieties is a common form of food fraud. GC×GC-TOFMS was used to investigate this fraud by characterizing and differentiating edible oils and edible oil mixtures. The chromatographic resolution of GC×GC and mathematical deconvolution of TOFMS were particularly useful for isolating individual analytes within these complex samples in order to distinguish the varieties. These capabilities can be used for authentication and also for other quality screening.



**Figure 8.** Representative GC×GC-TOFMS TIC chromatograms for a selection of edible oils. The visual 2D traces are distinctly different between the oil varieties and allow for rapid comparisons.



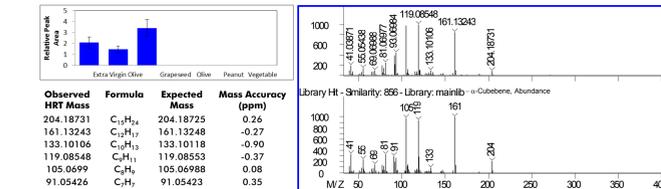
**Figure 9.** GC×GC can often chromatographically resolve analytes that coelute in the first dimension. In a given GC separation, octane and hexanal perfectly coelute (exceeding mathematical deconvolution capabilities) for an extra virgin olive oil sample. The associated GC mass spectrum is the combination of both analytes. With a comparable GC×GC separation, the analytes chromatographically separate in the second dimension, and each mass spectrum was isolated, allowing the analytes to be successfully identified.



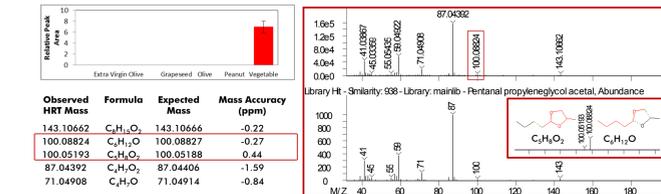
**Figure 10.** Even with GC×GC, coelutions can still occur and deconvolution can often mathematically resolve these. 1-penten-3-one and 1-penten-3-ol appear to be a single peak in the GC×GC data. Two distinct peak shapes are apparent by unique ions and the isolated mass spectra were library matched with similarities of 824 and 861, respectively.

## Sample-Distinguishing Analytes

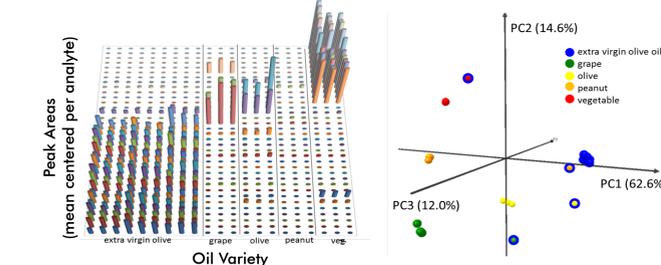
These analytical techniques isolated hundreds of individual analytes and feature selection tools were used to determine those that would distinguish the oil varieties. These and other class-distinguishing analytes were submitted to PCA to classify and differentiate the edible oil samples and mixtures. The accurate mass information from the GC-HR-TOFMS data allowed for further confirmation of analyte identity in many cases, and two representative examples are shown.



**Figure 11.** Accurate MS, acquired with GC-HRT, provides confidence in the analyte ID for α-cubebene, an analyte with herbal odor properties indicative of extra virgin olive oil.



**Figure 12.** Accurate MS, from GC-HRT, provides confidence in the analyte ID for pentanal propyleneglycol acetal, an analyte with nutty odor properties present in vegetable oil.



**Figure 13.** A collection of analytes that differ between oil varieties are shown and were submitted to PCA. The samples cluster in the PCA scores plot by variety with simulated fraud mixtures (denoted with blue outer circles) falling between each pure variety.

## Conclusions

This poster has demonstrated the broad applicability of GC and TOFMS in the food and beverage industry with highlighted examples from edible oils, hops, and beer. HS-SPME was used to pre-concentrate and collect volatile and semi-volatile analytes from each sample for analysis by LECO’s Pegasus 4D, HT, and/or HRT. This type of information provides food “omics” insight at various stages throughout production, including differentiation of raw materials, process changes, and finished products. Process changes were determined by monitoring flavor analytes from hops through a simulated boil in the beer brewing process with distinct time-dependent trends observed, including a decrease in essential oil levels corresponding to an increase in the duration of the boil. Beer samples were characterized and differentiated with specific analyte differences determined. Food fraud was also investigated with the analysis and characterization of edible oils and edible oil mixtures. The mixtures were clearly distinguished from the pure varieties by PCA with scores falling between those of the pure varieties used in the mixtures. These tools are very powerful and have broad applicability in the food and beverage industry.