Aroma Profile of Pet Food by GC-TOFMS and GCxGC-TOFMS

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1. Introduction

Monitoring the flavors associated with food products ensures consistency in flavors and provides good quality control since modifications to the flavor profile of a food item with natural and/or artificial flavoring can alter and enhance food products. Flavor profiles are comprised of the volatile and semi-volatile compounds that contribute to the characteristic aroma of a food item. These tend to be complex, because a large number of compounds can be involved and analyte concentration does not necessarily correlate with odor contribution. For these reasons, both good resolution and a wide dynamic range are required to adequately monitor and measure individual compounds in a food product that impact flavor.

This application note reports a method that isolates aroma and flavor compounds associated with pet food samples. Individual analytes were measured to compare flavors and to facilitate characterization of the samples. Sampling of the volatile and semi-volatile compounds was achieved with headspace solid phase micro-extraction (HS-SPME). The compounds were separated and detected with GC-TOFMS and with GCxGC-TOFMS.

2. Results and Discussions

GC-TOFMS

These methods provided good characterization of aroma compounds in pet foods. Monitoring individual analytes within the complex aroma profile was readily accomplished with the instrumentation and analysis software. Individual analytes were isolated through chromatography and with mass spectral deconvolution. With complex samples such as these, some analytes are likely to have similar properties, and regions of chromatographic overlap are often present. In these cases, overlapped analytes can often be isolated based on differences in mass spectral patterns with deconvolution algorithms, such as ChromaTOF® software's automated True Signal Deconvolution®.

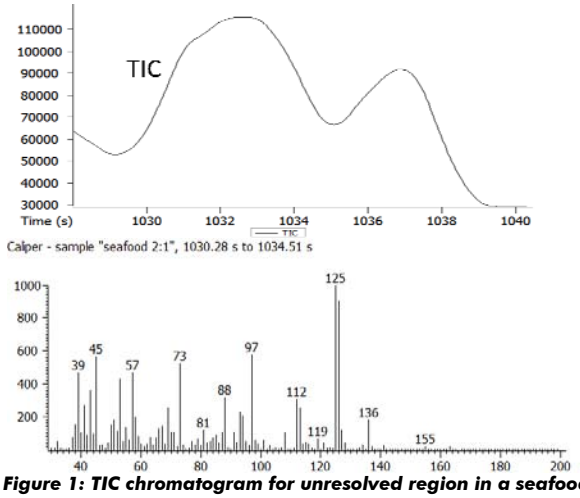

Figure 1: TIC chromatogram for unresolved region in a seafood-based sample and mass spectral data corresponding to the first apparent peak. It appears that two peaks are present in this retention window. The mass spectral data across the width of the first apparent peak has no reliable match to library spectra.

The deconvolution algorithm utilizes the full mass range data acquisition to isolate three analytes eluting in the first apparent peak, as shown in Figure 2. The second apparent peak in the TIC view is also comprised of three unique analytes, not shown for brevity. These analytes are mathematically resolved based on the mass spectral patterns across the width of the peak. When m/z unique to the overlapped analytes (88, 125, and 112) are plotted, all three peak shapes can be observed. Deconvolution provides mass spectral information and peak profile information for each coeluting analyte which can be used for identification and quantification. Quantification is based on peak area or height using unique m/z and identification is based on mass spectral matching of the pure spectra to library standards.

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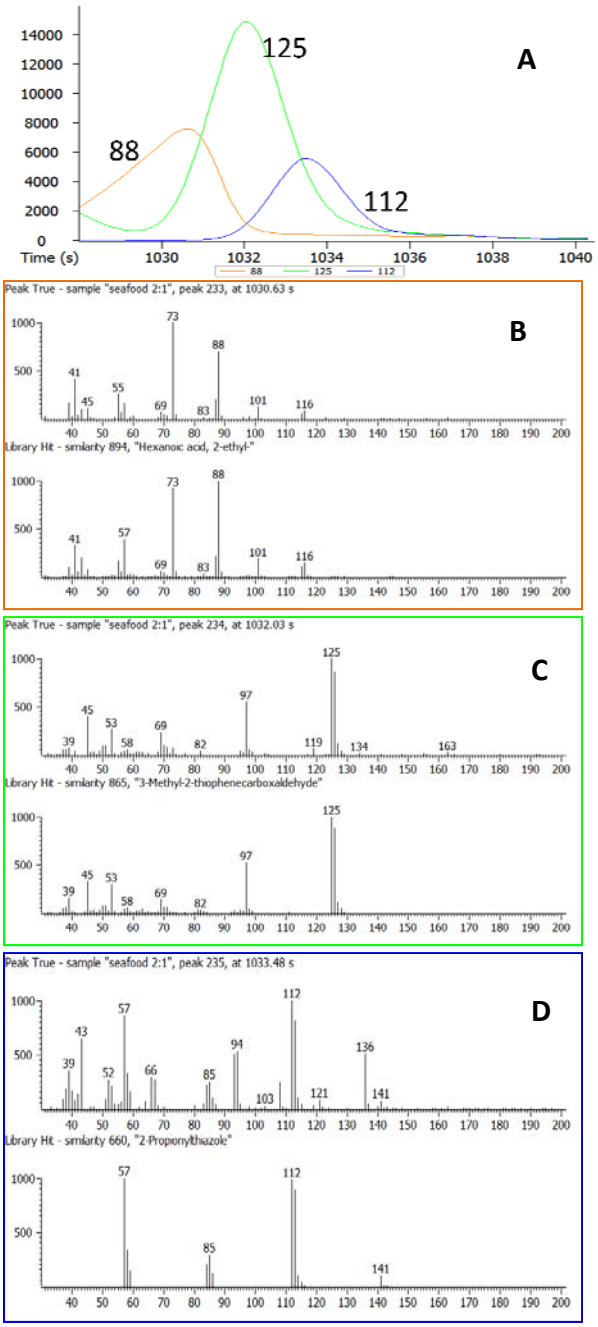


Figure 2: Deconvoluted spectra and peak profiles for the unresolved region in Figure 1. MS deconvolution isolates the overlapped analytes for identification. Of note, the unique m/z used for quantification are each present only in their respective deconvoluted spectrum.

The three coeluting spectra, shown in Figure 2B-2D, combine to the raw mass spectrum in Figure 1. Library matching of the peak true spectra offers identification information, summarized in Table 1. These analytes are known to be present in ingredients commonly used for pet food. Both 2-ethyl hexanoic acid, in Figure 2B, and 3-methyl-2-thiophene carboxaldehyde, in Figure 2C, match well to library spectra. The peak true spectrum for 2-propionylthiazole, in Figure 2D, however, contains several m/z that are not in the library match. This, along with the low match value, suggests that either identification is incorrect or that an overlapping analyte is still interfering. In some instances of chromatographic overlap, the mass spectral information can be deconvoluted, but in others the algorithm is unable to mathematically separate the analytes due to complete coelution. In these cases, unresolved analytes can sometimes be chromatographically separated with an additional separation dimension, utilizing GCxGC.

Table 1. Analytes deconvoluted in Figure 2

Analyte Name	Match Value	Unique Mass	Naturally present in:
2-ethyl hexanoic acid	894	88	lamb
3-methyl-2-thiophene carboxaldehyde	865	125	beef
2-propionylthiazole	660	112	lard

GCxGC

GCxGC can be beneficial in the analysis of complex samples as it offers improved peak capacity with two complementary separations and low-level detection through cryogenic focusing of thermal modulation. Both of these lead to a greater number of measureable analytes, thus more insight to complex samples. These benefits can be observed in the region of the GCxGC chromatogram corresponding to the GC separation shown in Figure 1. A contour plot is shown in Figure 3. The first dimension separation is displayed along the x-axis and the second dimension separation is displayed along the y-axis. The three previously overlapped analytes are now chromatographically resolved in the second dimension and a fourth analyte is also chromatographically resolved. The asterisk at the top of the chromatogram indicates 3-methyl-2-thiophene carboxaldehyde and the asterisk at the bottom indicates 2-ethyl hexanoic acid. What previously was mass spectrally deconvoluted as 2-propionylthiazole has now been chromatographically separated into 2-propionylthiazole and 2-acetyl-3-methylpyrazine.

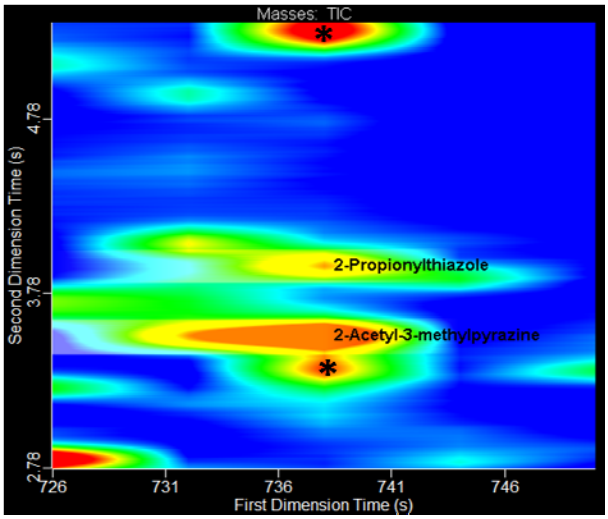


Figure 3. GCxGC data corresponding to the GC separation shown in Figure 1.

The mass spectra for the two overlapped analytes that were unable to be deconvoluted with GC-TOFMS, but are chromatographically resolved by GCxGC are provided in Figure 4. The spectrum shown in Figure 4A is the combined spectrum that was obtained with deconvolution of the GC separation. With the additional chromatographic resolution, the match value for 2-propionylthiazole has improved from 660 to 891 and the isolated spectrum is shown in Figure 4B. Additionally, information on 2-acetyl-3-methyl pyrazine, with a match value of 860, shown in Figure 4C, is now available. 2-acetyl-3-methyl pyrazine is an aroma compound that is naturally present in meat, pork, seafood, and potatoes and has roasted, nutty, and vegetable odor characteristics.

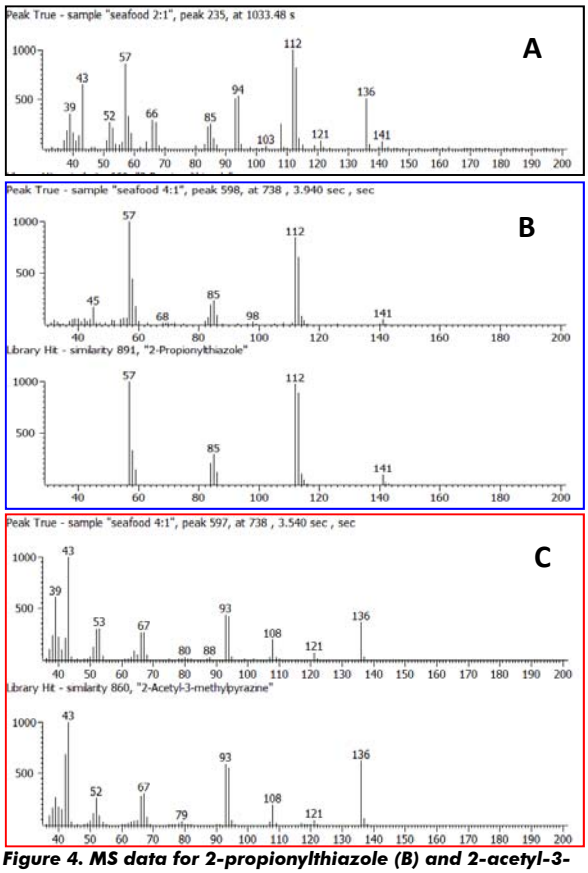


Figure 4. MS data for 2-propionylthiazole (B) and 2-acetyl-3-methylpyrazine (C) that have been chromatographically resolved with GCxGC.

Comparison of Flavors

With the ability to isolate individual analytes, these methods allowed for the comparison of various pet food samples to determine similarities and differences in the associated aroma profiles. The ChromaTOF software compiles the identification and quantification information for all peaks into Peak Tables for user review. A total of 491, 421, and 598 peaks were detected with S/N ≥ 200 in the GC-TOFMS seafood-, turkey-, and beef-flavored pet food samples, respectively. With GCxGC-TOFMS, the detected peaks increased to 1199, 957, and 1069, respectively. The increase in measured peaks can be attributed to both the improved peak capacity with the second column and the enhanced detection with thermal modulation. The Peak Tables contain many analytes associated with the ingredients commonly used in pet food and many that have aromas and flavors commonly associated with savory or meat-flavored food products. Clear differences were readily observed between pet food flavors. Chromatograms for each flavor are shown in Figure 5 and representative analytes are included in Table 2.

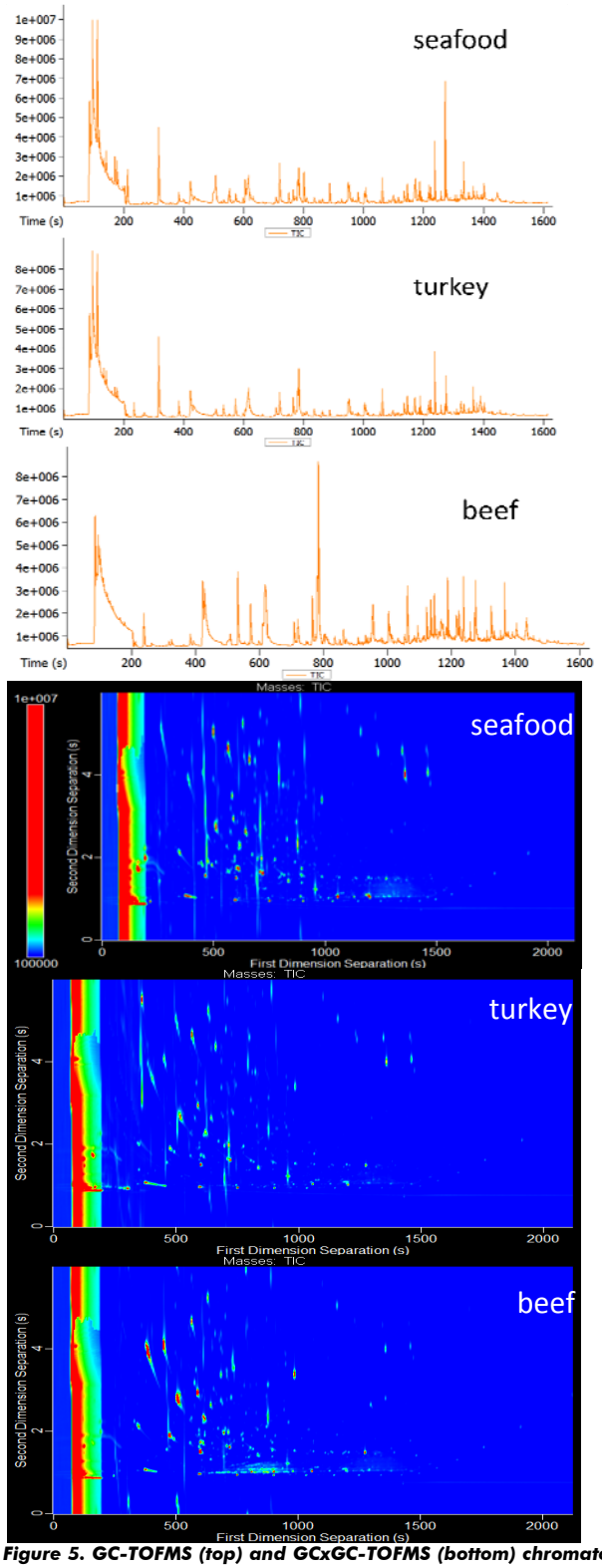
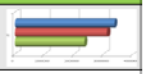
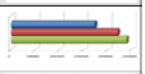
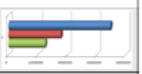
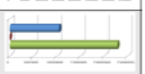
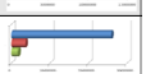
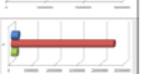
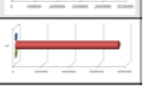


Figure 5. GC-TOFMS (top) and GCxGC-TOFMS (bottom) chromatograms for the seafood-, turkey-, and beef-based pet food flavors.

Table 2. Comparison of Flavors for Representative Analytes. Peak areas are shown in Blue = Seafood, Red = Beef, and Green = Turkey.

Analyte Name	Match Value	Flavor Notes	Naturally present in	Relative Intensity by Flavor
Nonanal	923	Potato, nutty	Beef, fish, and rice	
2-acetylthiazole	963	Corn, chip	Beef, pork, and shellfish	
Sulfurol	899	Meaty, brothy, roasted	Beef	
4-methyl thiazole	781	Vegetative, meaty, alliaceous	Beef, shrimp	
methyl-pyrazine	951	Nutty, brown, and roasted	Beef, chicken, fish	
3-methylthio propanal	907	Beefy, brothy, seafood nuances	Chicken and Ham	
2-methyl butanal	856		Fish	
2-ethyl furan	932	Earthy and malty	Beef, chicken, pork, and soy	
1-(2-furanyl)-ethanone	929	Nutty and Roasted	Beef, pork, and potato	
trimethyl pyrazine	925	Raw, nutty, potato	Beef	

3. Conclusions

The experiments described in this application note demonstrate a food, flavor, and fragrance analysis for the characterization of the aroma profile for pet food. HS-SPME was used to pre-concentrate volatile and semi-volatile compounds and LECO's Pegasus HT GC-TOFMS and Pegasus 4D GCxGC-TOFMS efficiently separated, quantified, and identified analytes within the complex sample matrix. Full mass range data acquisition with TOFMS allows for deconvolution of chromatographically overlapped analytes in many instances. When deconvolution is unable to resolve overlapped analytes, an additional chromatographic separation with GCxGC can often provide the required resolution to measure individual analytes within a complex sample.

4. Sample Preparations

Three pet food samples, seafood-, beef-, and turkey-based flavors, were prepared for analysis by combining 1.5 g of sample with 1.5 g of a saturated salt solution. Each sample was mixed in a 20 mL glass headspace vial that was sealed with a septum cap, prior to SPME analysis.

Gerstel's MPS2 Auto Sampler was used to automate the SPME sampling through LECO's ChromaTOF software. Immediately prior to extraction, samples were incubated at 53°C for 10 min. Extraction was accomplished by exposing a divinylbenzene/carboxen/polydimethylsiloxane (50/30 µm DVB/ Carb/ PDMS) SPME fiber (Supelco, Bellefonte, PA, USA) to the sample headspace for 40 min at 53°C. The fiber was then exposed in a 250°C GC-inlet for 2 min to desorb analytes for injection.

5. Experimental Conditions

GC and GCxGC Conditions

GC analyses were performed with LECO's Pegasus HT consisting of an Agilent 6890 GC equipped with a GERSTEL MPS2 Auto Sampler and LECO Pegasus TOFMS. GCxGC analyses were performed with LECO's Pegasus 4D consisting of an Agilent 7890 GC equipped with a GERSTEL MPS2 Auto Sampler and LECO's dual stage quad jet thermal modulator, secondary oven, and Pegasus TOFMS. Experimental conditions are listed in Table 3.

Table 3. Experimental Conditions

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)
Temperature Program	4 min at 35°C, ramped 5°C/min to 100°C, ramped 25°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 @ 1.5 ml/min (controlled via pressure ramps)
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)
Temperature Program	4 min at 35°C, ramped 10°C/min to 250°C, held 10 min; Secondary oven maintained +10°C relative to primary
Modulation	6 s with temperature maintained +15°C relative to 2nd oven
Mass Range	30-400 m/z
Acquisition Rate	100 spectra/s
Source Temp	250°C