Application Note

Instrument: Pegasus® BT 4D

Comprehensive Evaluation of Pesto Aroma Profiles Using SPME-GCxGC-TOFMS

Enhanced Aroma Characterization

LECO Corporation; Saint Joseph, Michigan USA

Key Words: Non-Target, Food, Aroma, GCxGC, TOFMS, Differentiation, Pesto, Foodomics

Introduction

The origins of pesto date back to ancient times. Although the main ingredients are well known (typically based on olive oil, basil, and pine nuts), the recipes of numerous commercially available pesto products can strongly differ. The aroma profile of the pesto is highly influential on consumers' perceptions and preferences. Thus, its examination plays an important role in definition of the "right" recipe, product stability, and product quality. The ability to comprehensively analyze and characterize pesto recipe variations allows for more optimal product development and therefore higher brand awareness and customer satisfaction.

There are a high number of taste and aroma-critical chemicals in pesto products that are present at a variety of concentrations. A number of low level but high aroma potency species often coelute with numerous other species, including species present at high concentrations. It can therefore be difficult or impossible to separate and accurately detect them with conventional one dimensional GC-MS approaches. In this study, we evaluate the use of comprehensive two-dimensional GCxGC-TOFMS to significantly improve the separation, detection, and therefore identification of species in pesto products to provide a significantly higher quality and more informative characterization ability.

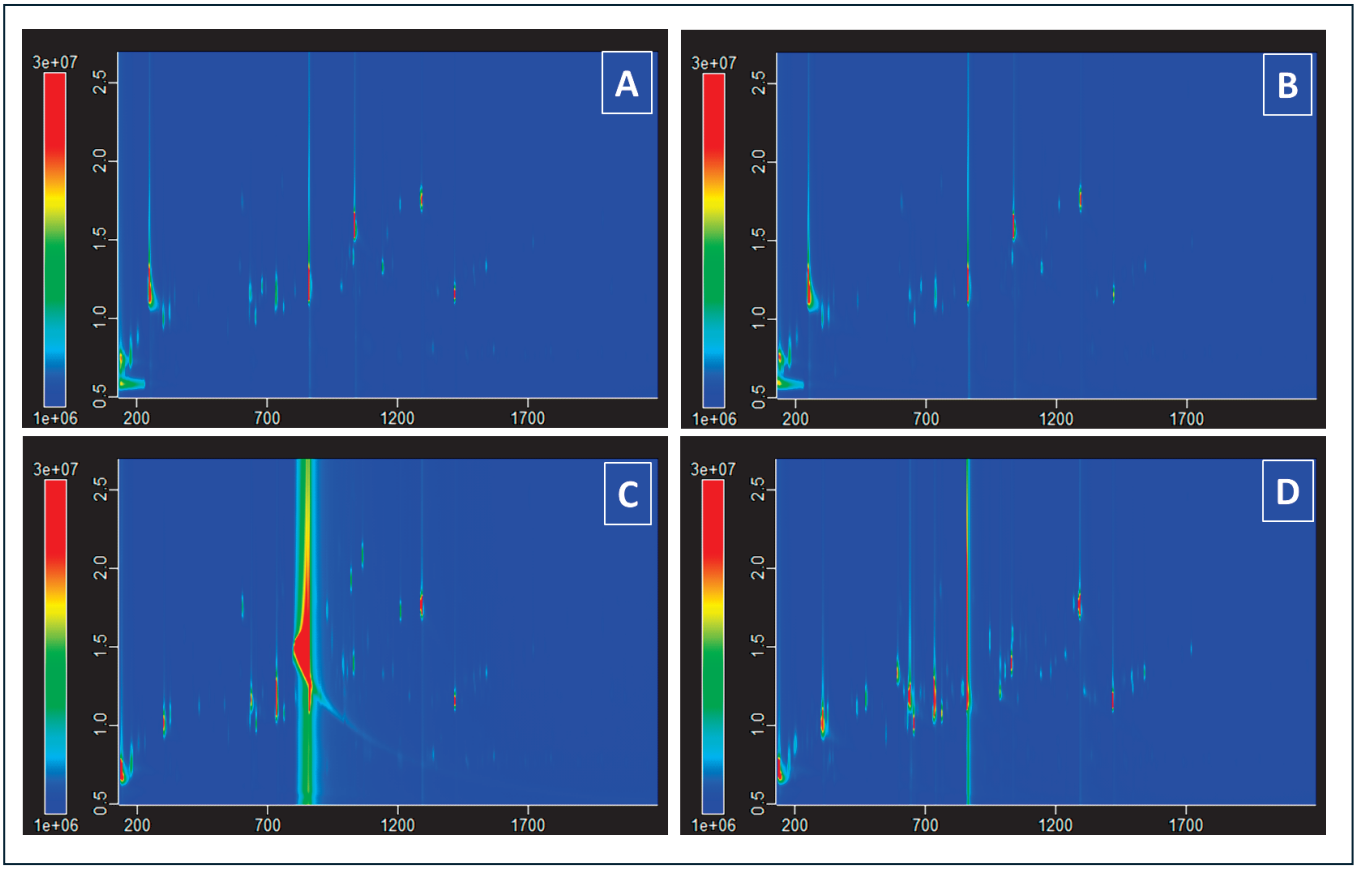

Figure 1: Representative TIC contour plots of four pesto samples.

Experimental

Materials

Four commercially available pesto products from different producers were obtained to allow method optimization and a representative evaluation of typical pesto aroma profiles.

n-Alkane standards (C7-C30), obtained from Restek were diluted to 10 ppm and analyzed for calculation of linear retention indices.

Sample Extraction & Instrumental Conditions

The samples were prepared for HS-SPME by weighing pesto aliquots (1 g ± 0.1 g) into 10 mL vials sealed by septum caps. The sample incubation (15 min at 60 °C) was followed by extraction (15 min at the same temperature). Extraction was performed using a 1 cm DVB/CAR/PDMS fiber (Sigma Aldrich) which was then immediately desorbed in the GC inlet for analysis with conditions listed in Table 1.

Table 1. Pegasus® BT 4D GCxGC-TOFMS Conditions

GC	**LECO GCxGC QuadJet™ Thermal Modulator**
Injection	2 min fiber desorption with inlet temp. 220 °C , split 20:1
Columns	^{1}D: Rxi-5SilMS, 30 m x 0.25 mm i.d. x 0.25 µm coating (Restek)
	^{2}D: Rxi-17SilMS, 0.6 m x 0.18 mm ID x 0.18 µm coating (Restek)
Carrier Gas	He @ 1.2 ml/min
Oven Program	40 °C (2 min), ramp 5 °C/min to 210 °C, 20 °C/min to 280 °C
Secondary Oven	+ 5 °C (relative to the primary oven temperature)
Modulator	+15 °C (relative to the secondary oven temperature)
Modulation Period	2.7 sec
Transfer line	300 °C
MS	**LECO *Pegasus* BT 4D**
Ion Source Temperature	250 °C
Mass Range	40-550 m/z
Extraction Frequency	32 KHz
Acquisition Rate	200 spectra/s

Results and Discussion

Representative two-dimensional contour plots of four different pesto samples (A,B,C,D) are shown in Figure 1. The complexity of these type of samples is apparent with a high number of peaks visible in the TIC. One of the benefits of coupling GCxGC separations with TOFMS is that highly comprehensive non-target data is achievable due to extremely fast data acquisition rates over the whole mass range and at increased sensitivity due to the band focusing during thermal modulation. Compared with 1D GC and/or non-TOFMS approaches, richer non-target data with higher-quality mass spectra are obtained, allowing higher confidence in library matching for the discovery of a higher number of unknowns.

In more focus, Figure 2 shows >400 peaks observed and identified in sample A, where each black dot represents a found peak. In this particular case, only peaks with SN>20 and library similarity higher than 750/1000 (i.e. 75%) are shown. A high number of peaks of lower intensity and spectral match were filtered out. Identification was aided using retention index matching and automated removal of hits outside a given RI window based on the NIST MS Library.

The extremely narrow peaks (with typical FWHH of 60-80 ms achieved in this case study) formed during the thermal modulation process used between the primary and secondary columns during comprehensive GCxGC, require fast MS detection. The *Pegasus* BT TOFMS detector allows high data acquisition speeds (up to 500 spectra/s), enabling the collection of sufficient data across each chromatographic peak and thus successful and automated advanced peak finding via spectral deconvolution algorithms.

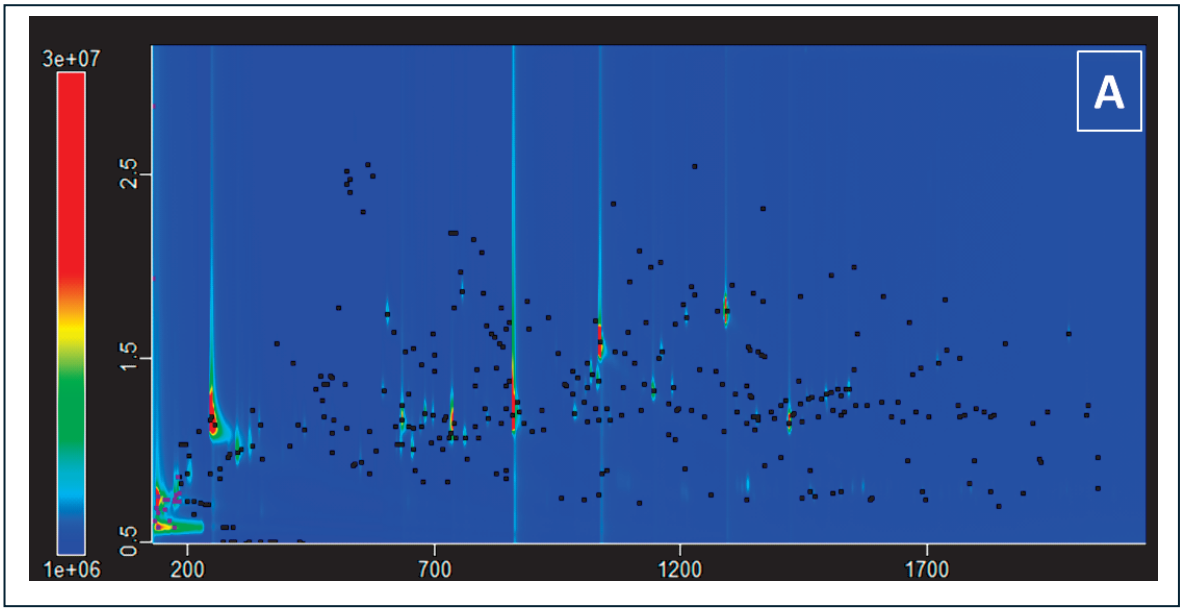

Figure 2: 418 Found Peaks in Sample A (SN>20, similarity >750).

Examples of the enhanced chromatographic resolving power of GCxGC technology over traditional one-dimensional separations are shown in Figure 3 and 4. Here, the chromatographic separation seen in the y-axis of the contour plot (i.e. polarity separation) is clear. It can be seen that in a one-dimensional separation, the two components would completely coelute, having identical 1D retention times (x-axis), while they are sufficiently resolved thanks to the 2nd dimension column separation, so both peaks could be correctly found, their spectra successfully deconvoluted and excellent spectral similarities achieved.

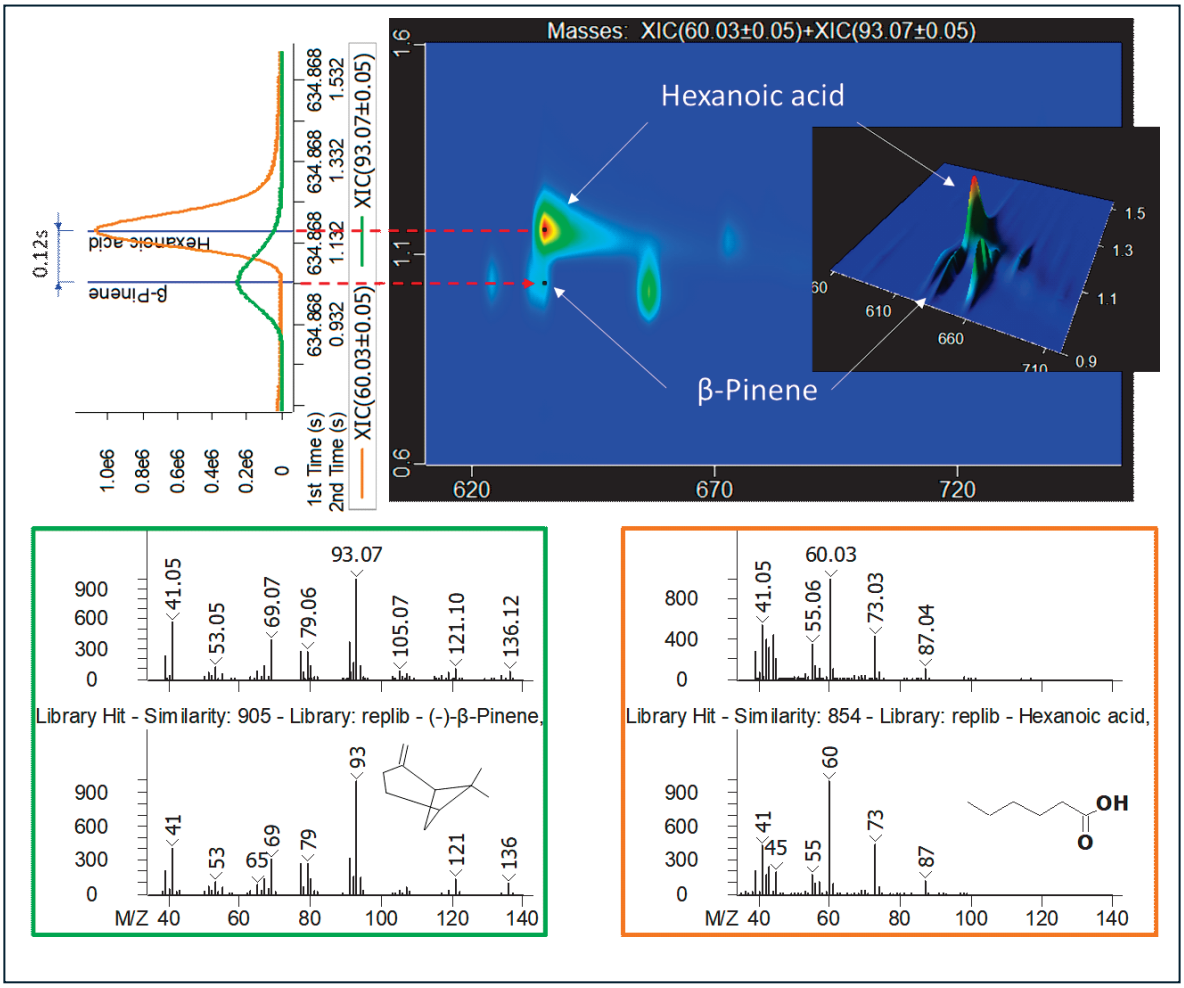

Figure 3: Example of the enhanced resolving power of GCxGC for β-Pinene and Hexanoic acid.

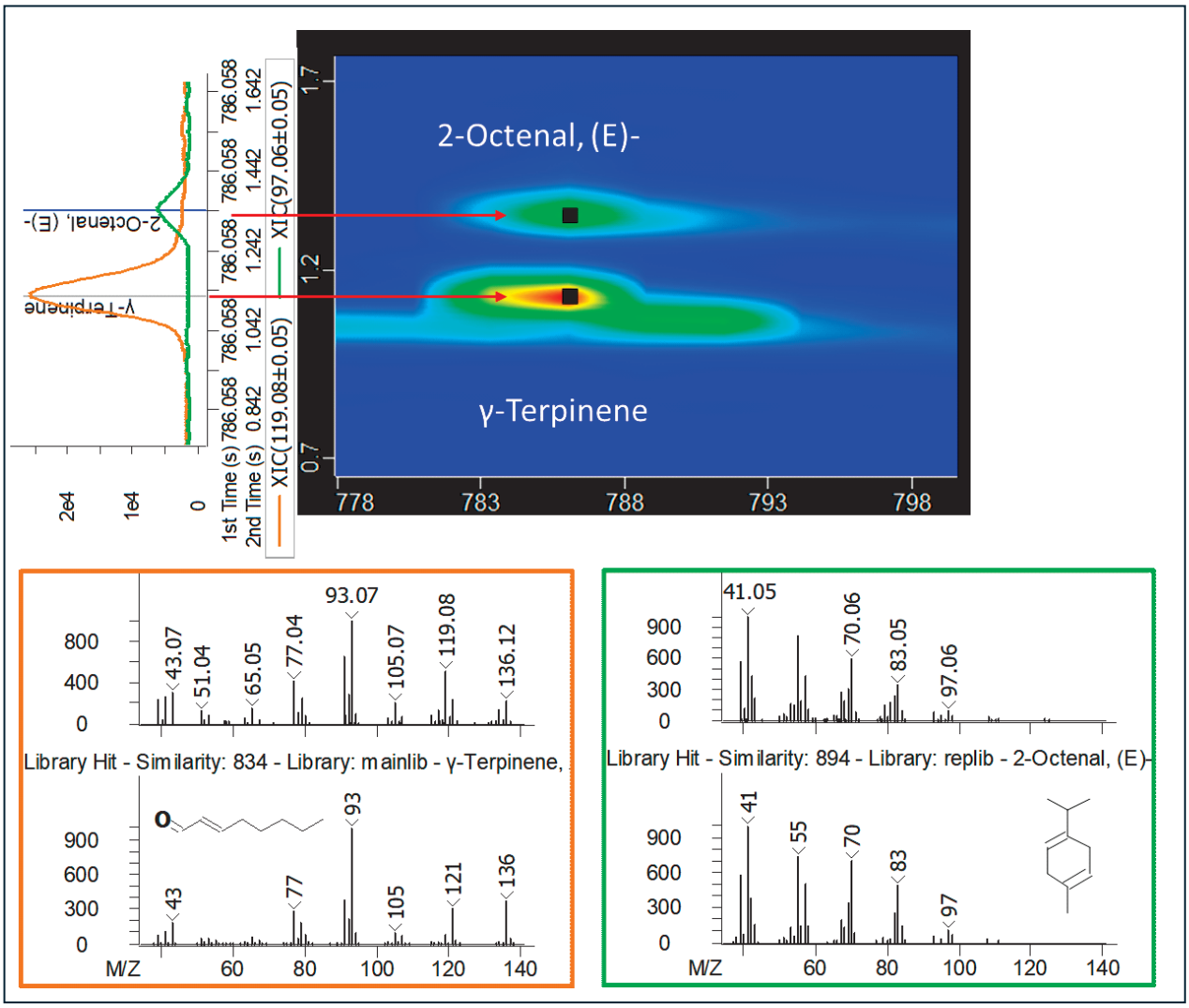

Figure 4: Example of the enhanced resolving power of GCxGC for 2-Octenal (E)- and γ-Terpinene.

In the examples above, the correctly found and deconvoluted peaks' apexes were well separated from neighboring entities. For even more closely eluting and even almost fully coeluted peaks, as shown in Figure 5a, the combination of high resolving GCxGC separation and fast data acquisition speeds is critical, allowing efficient spectral deconvolution to resolve differences in analyte species from chemical noise and background (e.g. GC column bleed) and/or coeluting component spectra, even though the observed peak apeces were only 0.025 s apart.

The benefits of LECO's deconvolution algorithm, resulting in successful peaks' identification is demonstrated in Figure 5b comparing (i) raw spectra with (ii) deconvoluted ones, and (iii) library hits for both components.

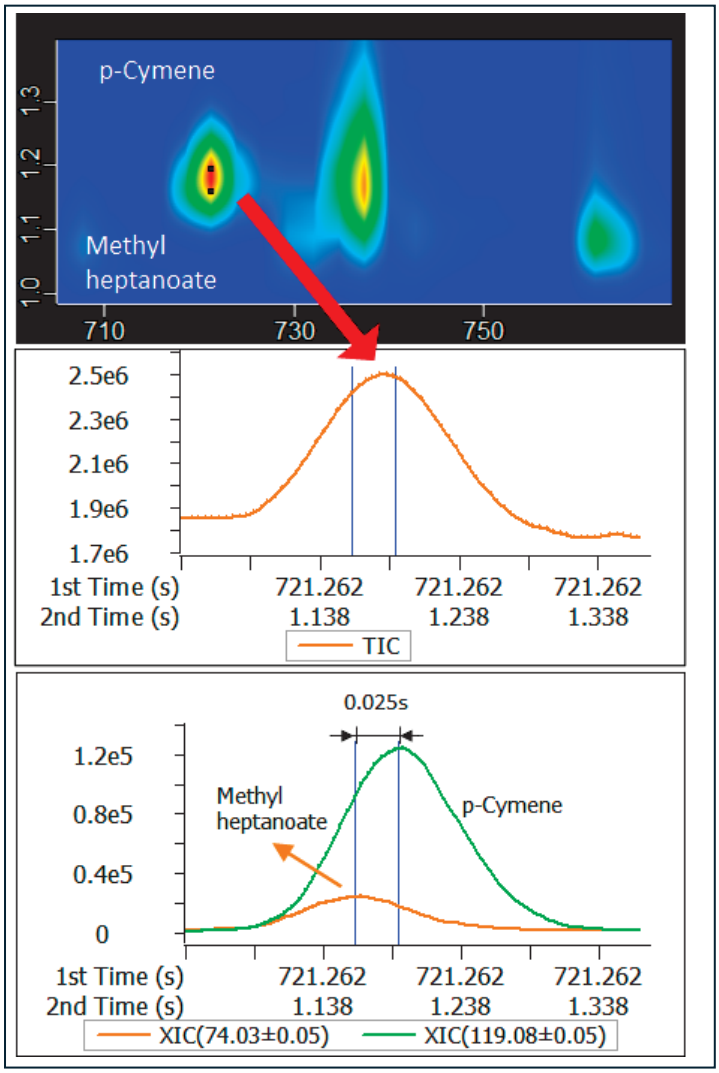

Figure 5a: Example of the efficiency of LECO's spectral deconvolution and Peak Find algorithms demonstrated on closely coeluting peaks.

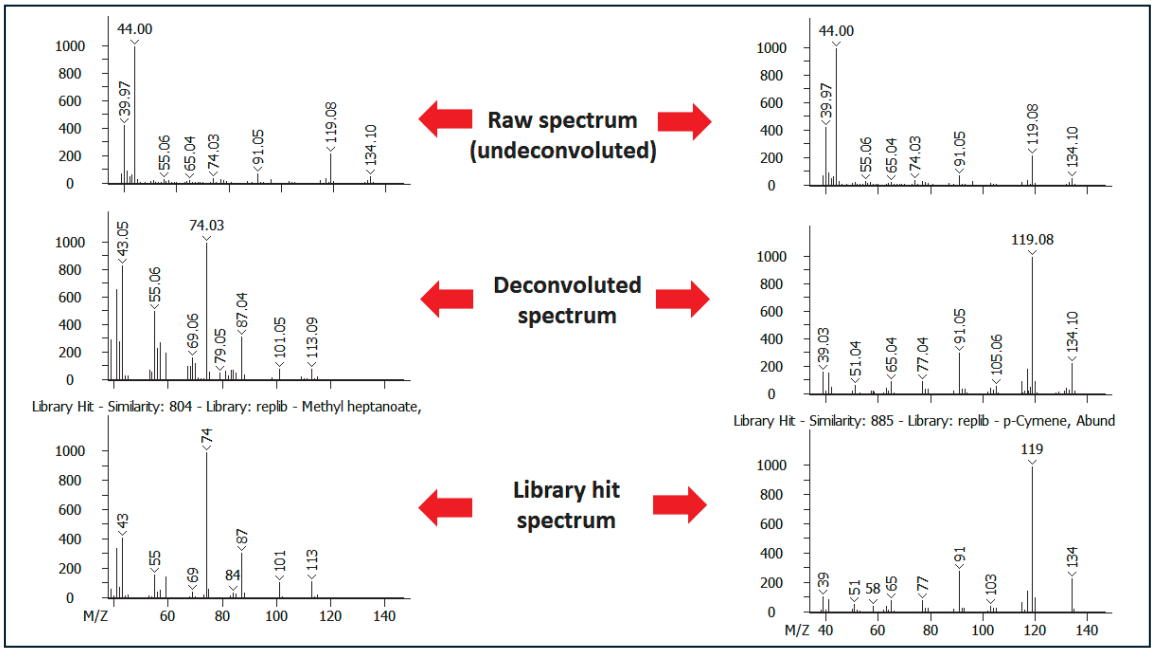

Figure 5b: Benefits of highly effective spectral deconvolution clearly show presence of both methyl heptanoate and p-cymene.

These examples of using GCxGC for complex sample analysis clearly show how many more analytes can be uncovered and identified, compared with using traditional single-dimensional GC separations and non-TOFMS detection, which typically struggle to provide sufficient data quality. Of course with higher quality and richer data, the comparison and differentiating of samples based on hundreds of individual analytes in each sample could be another demanding task. One of the approaches for sample comparisons can be visual evaluation of TICs (as shown in Figure 1). A more targeted way is to compare profiles of samples on known masses (e.g. m/z 93 or 121 for terpenoic compounds), as demonstrated in Figure 6. This zone can be then zoomed in comparing a particular component in different samples and its area/intensity in particular, as shown in Figure 7, where 3D plots of α-Pinene are visually compared.

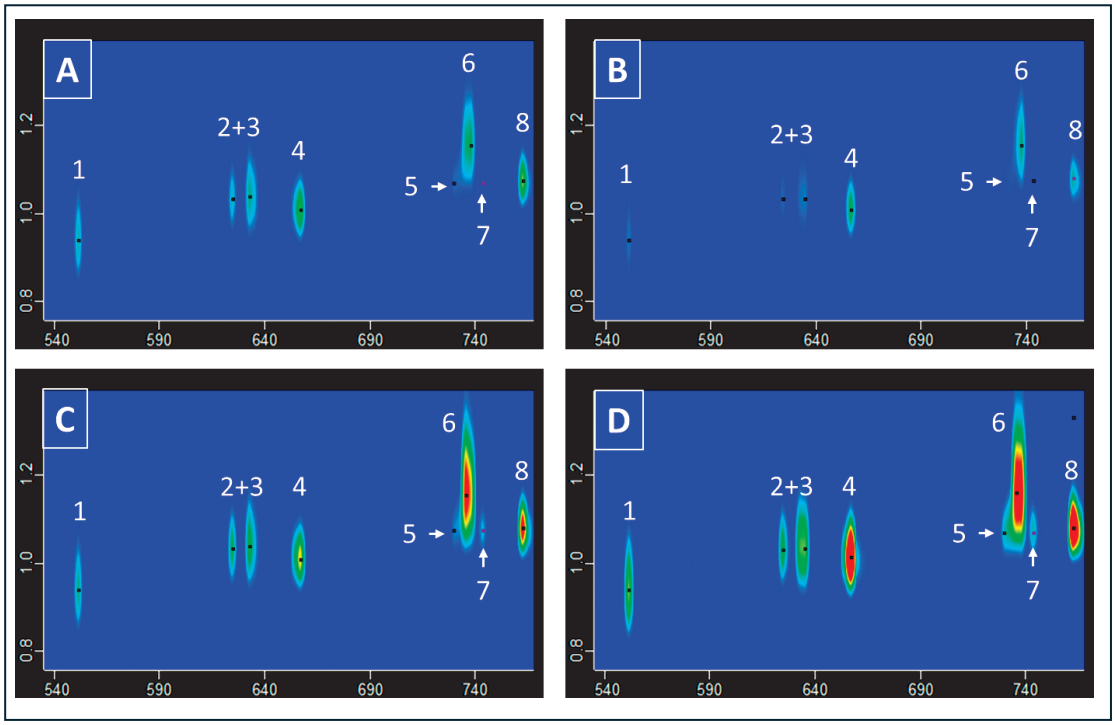

Figure 6: Semi-targeted visual comparison of GCxGC plots on zoomed terpenoids band. Individual peak identification is based on NIST MS library search and refined by retention indices. Visualized at m/z 93.06 ± 0.05. 1- α-Pinene, 2+3 - β-Pinenes, 4- β-Myrcene, 5- D-Limonene, 6- Eucalyptol, 7- trans-β-Ocimene, 8 - cis-β-Ocimene.

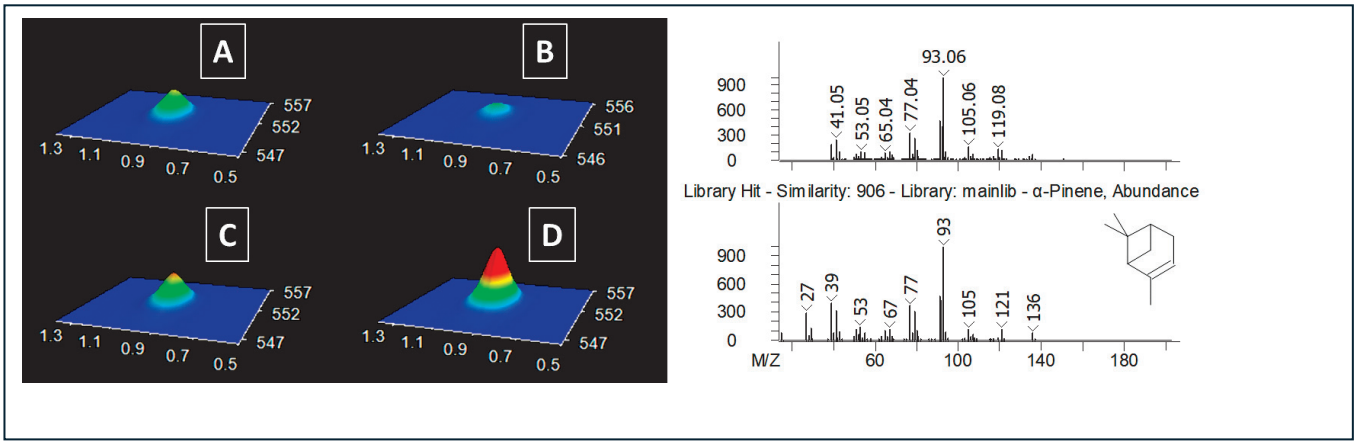

Figure 7: 3D plots of α-Pinene in four compared samples are shown along with its library matched spectra. Visualized at m/z 93.06 ±0.05.

Such semi-targeted approaches can of course traditionally be difficult to apply for hundreds of peaks found in multiple samples. One of the advantages of LECO's ChromaTOF® brand software is a feature called "References." This tool enables (i) comparison of selected peaks across a set of single or multiple samples, (ii) computation of relative concentration of analytes in a sample with respect to user-specified conditions, and (iii) classification of each chromatographic peak in four different categories. Each category indicates the relation to the reference, as shown in Figure 8. Analytes that are present in both the reference and the sample are tagged "Match" or "Out of Tolerance" depending on their relative peak intensity compared to a user-specified threshold. The "Not Found" category indicates analytes only present in the reference, while "Unknown" means that peak was located in the examined sample and not in the reference sample. This can be easily understood, for example with the Estragole peak (Figure 8). Sample D was defined as a reference to be automatically compared with all other examined samples (i.e. A, B, and C). Estragole was categorized as a "Match" in sample D (i.e. identical with defined reference and thus the RT, spectra, and area were fully corresponding with its reference). The same compound peaks were then classified as "Out of tolerance" in samples A and B, while in sample C, this particular peak was not found at all. The prevalence of estragole peaks in the analyzed samples are then captured by 3D plots and can be also demonstrated by overlaying the most abundant GCxGC slice on the linear chromatogram.

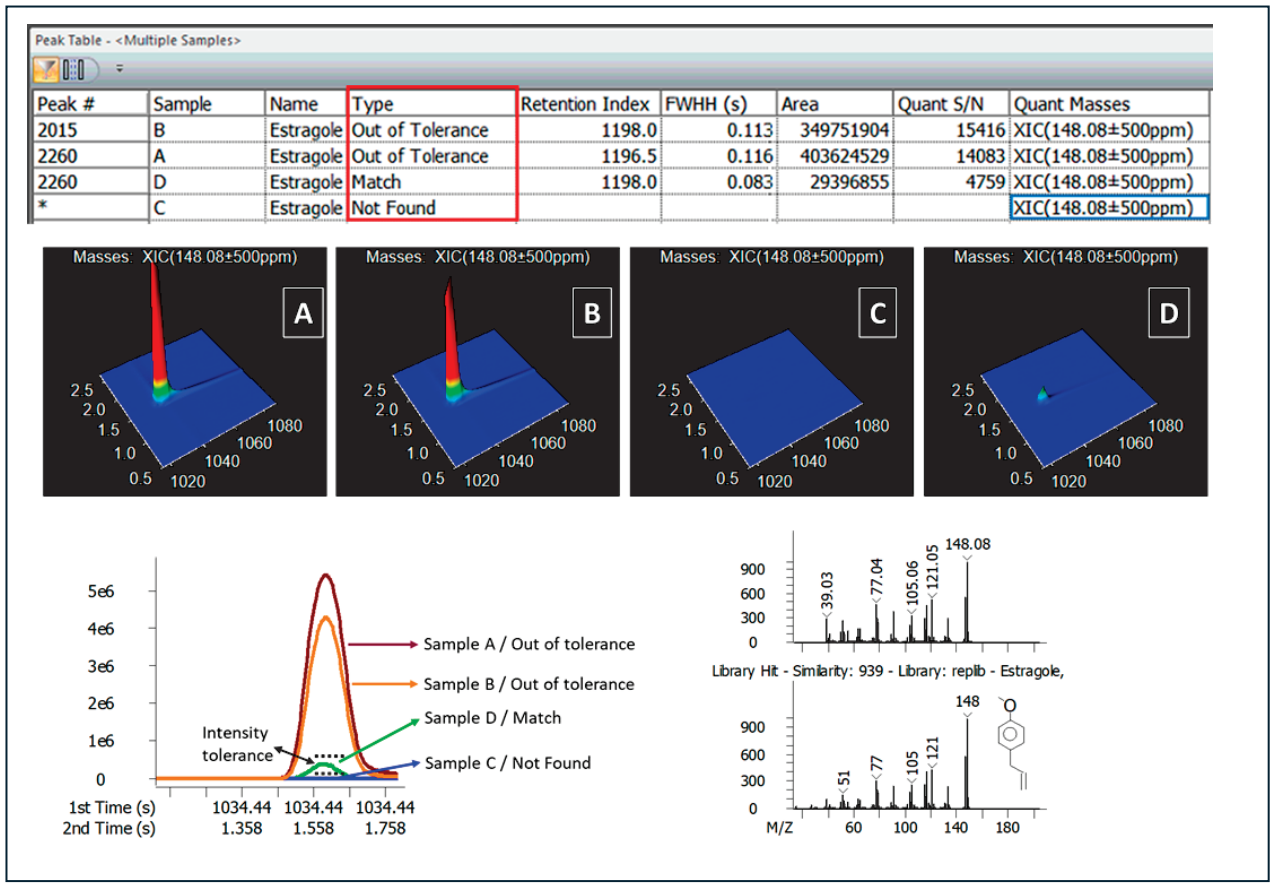

Figure 8: Example of the Reference feature in LECO's ChromaTOF SW. The automated comparison of four examined samples resulted in categorization of the Estragole peak as "Match" (sample D), "Out of tolerance" (samples A, B) and "Not-Found" (sample C). The peak intensity can be then also compared on (i) 3D plots of estragole and (ii) overlayed linear plots of the most abundant GCxGC slice.

A different example is described in Figure 9, where a peak of 1,2-Propanediol was found at high intensities (S/N >10000) in samples A & B therefore tagged as "Unknown" in their peak tables, since it was not found in the reference (i.e. Sample D where this particular peak was below S/N threshold and thus not imported into the reference).

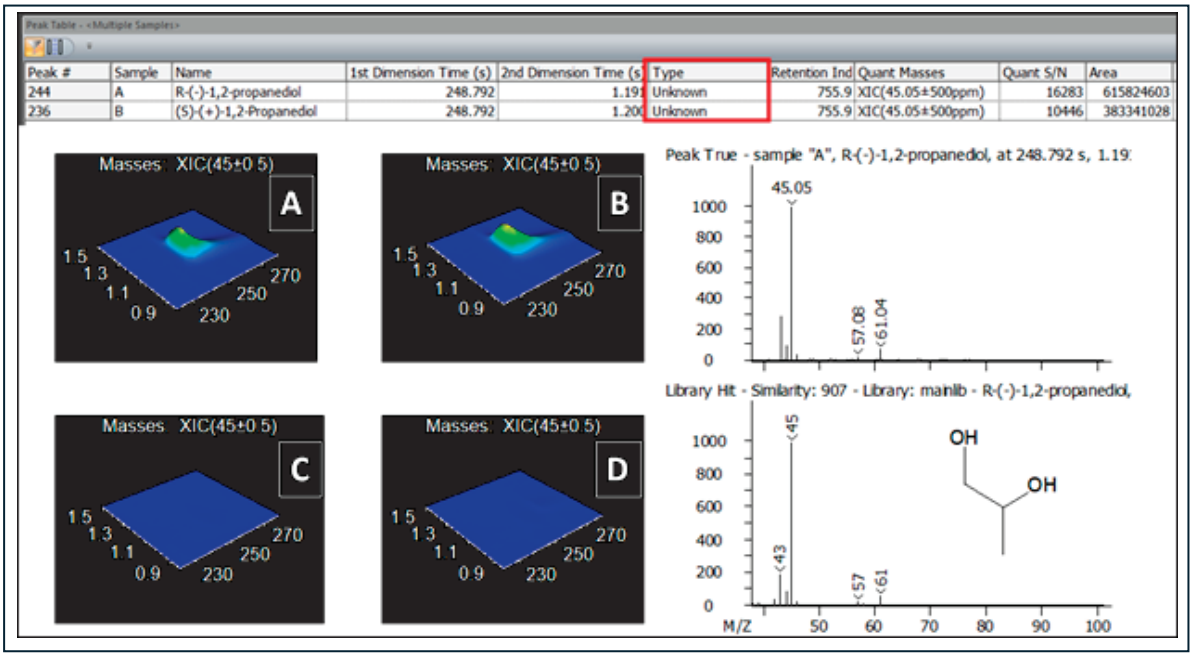

Figure 9: Example of 1,2-Propanediol peak that was not present in the reference (sample D) but found in samples A and B – tagged as "Unknown".

Conclusion

This study demonstrates how applying GCxGC-TOFMS and the analytical features built in LECO's *ChromaTOF* software deliver enhanced solutions for characterization of complex aroma samples. The *Pegasus* BT 4D is a powerful analytical tool that allows easy non-target GCxGC-TOFMS analyses to discover more in complex samples. In this work, variations of pesto samples were examined. The combination of HS-SPME sample introduction, GCxGC, and TOFMS allowed enhanced separation and detection of critical aroma species from each other, sample matrix interferences, and chromatographic noise, thus enabling an efficient identification for hundreds of analytes. Their identification by a traditional one-dimensional approach would not be possible. Deconvolution provided additional separation in instances of chromatographic coelution. The ease of use of LECO GCxGC-TOFMS hardware and benefits associated with *ChromaTOF* software enabled a time efficient and high-quality workflow to be applied, compared with a standard analysis. The utilization of the "Reference" tool was explored to demonstrate a differential analysis. This tool rapidly determined analyte similarities and differences between samples. A collection of representative differences was highlighted here, while many more were observed in this data. This data-analysis strategy offers an automated aroma profiling of analytes that are characteristic for each sample and/or sample type(s).

Authors

Tomas Kovalczuk*, Nick Jones*, Sebastiano Panto*, Michele Suman**
*LECO European Application & Technology Center, Berlin (Germany)
**Advanced Research Laboratory, Barilla G. e R. Fratelli S.p.A., Via Mantova 166-43122 Parma (Italy)
**Department for Sustainable Food Process, Catholic University of the Sacred Heart, 29121 Piacenza (Italy)

Additional Resources

Reference application note 203-821-642, where we evaluated a statistical comparison of a larger sample set of pesto products using a new software approach. We demonstrate how, using a novel, tile-based Fisher ratio approach, the quality and speed of determination and comparison of statistically significant differences can be improved.

LECO Corporation | 3000 Lakeview Avenue | St. Joseph, MI 49085 | Phone: 800-292-6141 | 269-985-5496
info@leco.com • www.leco.com | ISO-9001:2015 Q-994 | *LECO is a registered trademark of LECO Corporation.*
Pegasus, ChromaTOF are registered trademarks of LECO Corporation.

Form No. 203-821-641 2/22—REV0