Chemometric Assessment of Volatile Fraction of Pesto by SPME Arrow GC Orbitrap Mass Spectrometry

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Figure 1. Exactive GC Orbitrap GC-MS coupled with a TriPlus RSH autosampler.

ABSTRACT

"Pesto genovese' is a well-known pasta sauce. Pesto is a basil-based sauce characterized by unique organoleptic features associated with its ingredients, consisting mainly of crushed basil leaves, cheese (parmesan or pecorino), pine nuts and garlic blended with extra-virgin olive oil.

The production of pesto for wide distribution requires the use of additional ingredients and various technologies such as pasteurization and sterilization to extend the product shelf-life ensuring freshness for consumers. The preservation processes usually require high temperatures that can lead to changes in pesto composition affecting its taste and aroma.

In this study headspace solid phase micro-extraction (SPME) with Arrow technology coupled with gas-chromatography (GC) and Thermo Scientific™ Orbitrap™ high resolution mass spectrometry (HRMS) was used to determine the volatile profile of various pesto samples that were produced using various technological methods.

The SPME Arrow allowed for sample extraction and concentration in a single step, without the need of time consuming sample preparation and in a fully automated way. The improved geometry of the fiber provided a larger volume and a thicker coating phase allowing for fast extraction (15 minutes) of a large number of VOC ranging from major monoterpenes (like anethole, RT=13 min) to the less predominant ones (such as γ-terpinene, RT=9.56 min).

The study of the composition of the volatile fraction of pesto sauce ingredients (volatolomic profile) can help to discriminate among different production processes, for example γ-terpinene and linalool are predominant in heat-processed samples. Volatile compounds can be easily extracted and concentrated using the headspace solid phase microextraction (HS-SPME) technique while a confident detection of the compound can be achieved through the high resolution accurate mass Orbitrap technology coupled with gas chromatography. In this study the Orbitrap technology coupled with SPME Arrow extraction was used to assess the volatile profile of pesto sauce. Thermo Scientific™ Compound Discoverer™ 3.1 software was used for unknown compound deconvolution, identification, sample group assessment and multivariate statistical analysis. Principal component analysis (PCA) resulted in identification of the main components linked to the different production processes and that are responsible for the differences observed between the samples.

MATERIALS AND METHODS

Twenty industry manufactured pesto samples were prepared in triplicate by weighting 1.0 g from each sample and transferring it into a 10 mL crimp top headspace vial (vials P/N 10-CV, caps P/N 20-MCBC-ST3). Each jar was well mixed to homogenize the matrix before weighting. A blend (pooled sample group) was obtained by pooling together all the samples. In order to reduce the bias in the results the sample vials were analysed in randomly. A retention index mix (Sigma Aldrich, C7-C30 saturated alkanes, P/N 49451-U) was injected at the beginning of the sequence and used to derive the RI of chemical components putatively identified by NIST17 library following spectral deconvolution. In all experiments, a Thermo Scientific™ Exactive™ GC Orbitrap™ GC-MS equipped with two Thermo Scientific™ Instant Connect split/splitless SSL Injectors (SPME Arrow liner 1.7 mm ID, P/N 453A0415) was coupled with a Thermo Scientific™ TriPlus™ RSH™ autosampler with SPME Arrow configuration. Chromatographic separation was achieved on a Thermo Scientific™ TraceGOLD™ TG-1MS capillary column, 30 m × 0.32 mm × 1.0 µm (P/N 26099-2910). Additional HS-SPME Arrow and Orbitrap GC parameters are detailed in Table 1. The triple coating phase of the DVB/CWR/PDMS fiber (P/N 36SA11T3) allowed for effective extraction of a wide range of volatiles such as alcohols, aldehydes, ketones and esters. Data was acquired Thermo Scientific™ TraceFinder™ software. Compound Discoverer software was used for spectral deconvolution, compound identification and multivariate statistical analysis.

Table 1. HS-SPME Arrow and Exactive GC operating conditions for volatomic profile assessment of pesto samples.

| TriPlus RSH –HS-SPME Arrow Parameters	
Fiber:	SPME Arrow DVB/CWR/PDMS
Coating Phase Thickness (µm)	110
Coating Phase Length (mm)	20
Incubation Temperature (⁰C):	60
Incubation Time (min):	15
Incubation Speed (rpm)	500
Extraction Temperature (⁰C):	60
Extraction Time (min)	15
Stirring Speed (rpm)	1500
Fiber Depth in Vial (mm)	25
Fiber Depth in Injector (mm)	70
Desorption Time (min)	2
Analysis Time (min)	40
Inlet for Fiber Conditioning	
Fiber Pre-Conditioning Time (min)	0
Fiber Post-Conditioning Time (min)	15
Carrier Gas, Carrier Mode, Flow (mL/min):	He, constant flow, 6
Fiber Depth in Injector (mm)	**70**

Trace 1310 GC Parameters	
Inlet Module and Mode:	SSL, split
Split Ratio:	20:1
Carrier Gas, Carrier Mode, Flow (mL/min):	He, constant flow, 1.8
Oven Temperature Program:	
Temperature 1 (⁰C):	40
Hold Time (min):	2
Temperature 2 (⁰C):	150
Rate (° C/min):	10
Temperature 3 (⁰C):	260
Rate (° C/min):	5
Temperature 4 (⁰C):	300
Rate (° C/min):	25
Hold Time (min):	3
Total GC Run Time (min):	40

Exactive GC mass Spectrometer Parameters	
Transfer Line Temperature (⁰C):	280
Ion Source Temperature (⁰C):	280
Ionization Type:	EI/PCI
Electron Energy (eV)	70
Acquisition Mode:	full scan
Mass Range (Da)	50-550
Resolving Power (FWHM)	60,000 @ *m/z* 200 FWHM
Lockmass	207.03235

RESULTS

Component identification

Samples were acquired in full-scan mode at 60,000 FHWM resolving power and subjected to Compound Discoverer for Chemometric assessment and putative identification of unique features. Differences in the chromatographic profile of the samples were visible even in the TIC as demonstrated in Figure 2.

Figure 2. Total Ion Chromatogram (TIC) obtained for pesto samples. Differences in the volatile profile can be seen according to the suppliers.
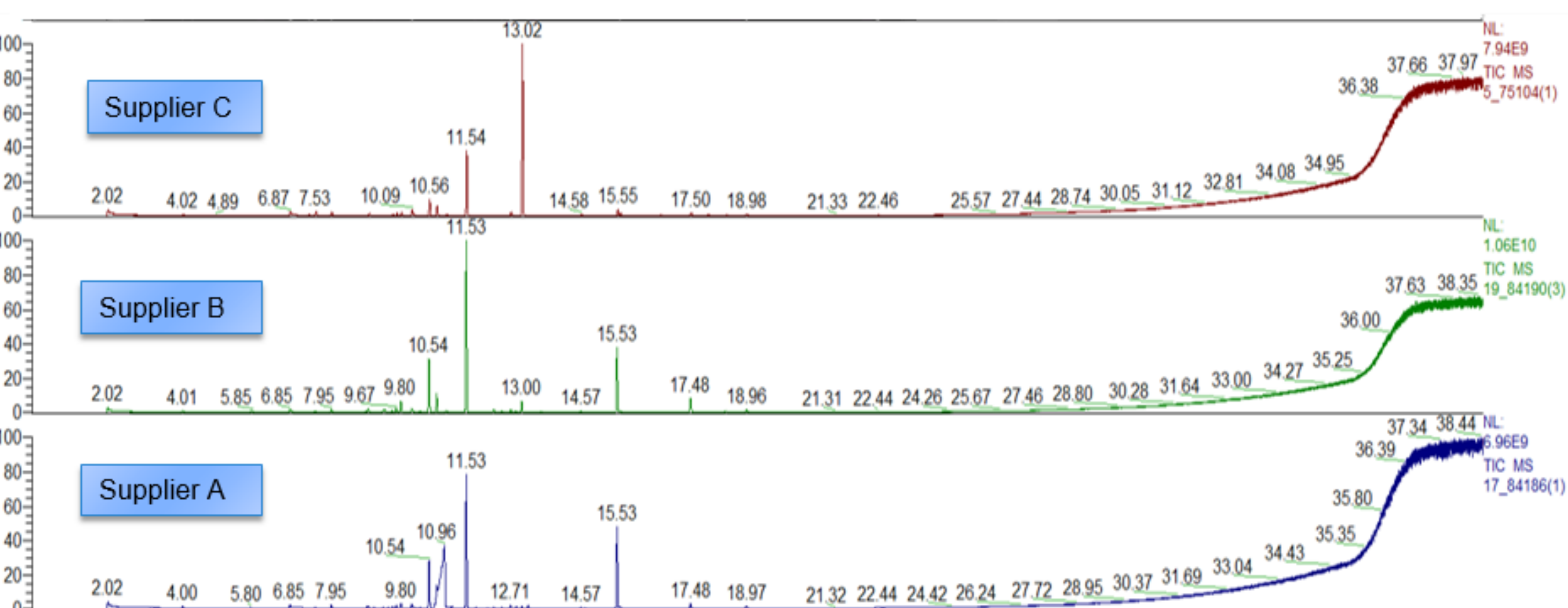
Although differences can be visually seen in the TIC comparison, it is essential that all features are extracted from the data and analysed statistically. Compound Discoverer was used to extract, deconvolute and identify the unknowns basing on mass based spectral library (NIST 2017). Compounds were scored based on the total score (derived from a combination of library search index score, high resolution filtering (HRF) and presence/absence of the molecular ions) as well as retention index difference from expected values. An example of such peak identification workflow is reported in Figure 3 for eugenol.

The wide dynamic range and the <1 ppm mass accuracy ensured the detection of compounds present at high and low concentrations. Moreover, at routine 60,000 resolution enough scans/chromatographic peak are obtained as shown for eugenol (*m/z* 164.08324, ±5 ppm mass window) in Figure 4.

Figure 3. Compound Discoverer result browser showing peak deconvolution results with eugenol as an example (RT=15.53 min, *m/z* 164.08325). Overlaid XIC (extracted ion chromatogram) of m/z 164.08325 corresponding to eugenol base peak ion in all samples analysed (A); results table with list of compounds detected and identified based on library search (B); deconvoluted EI spectrum of eugenol (C).
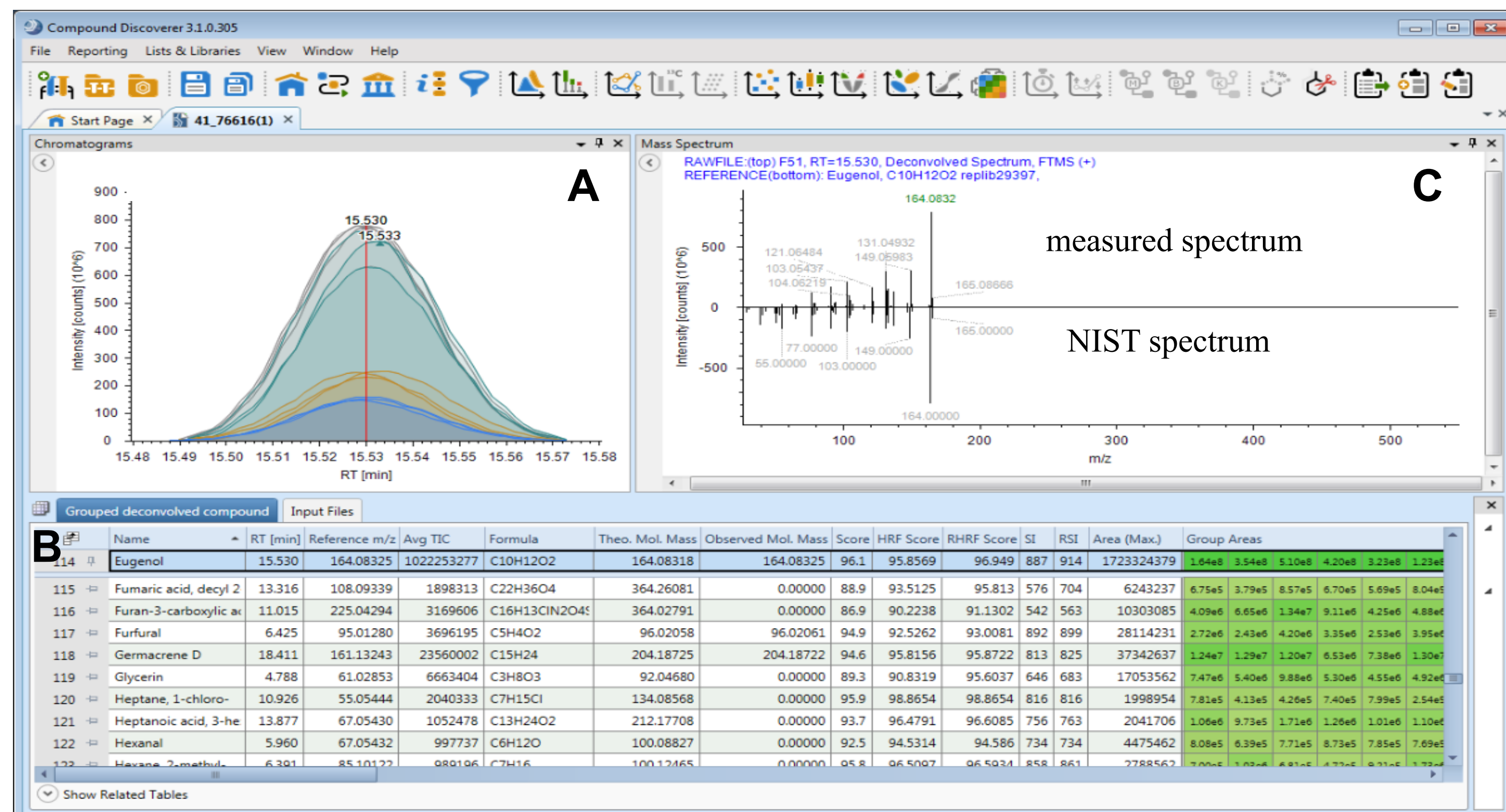

Figure 4. Extracted Ion Chromatogram (EIC) for eugenol *m/z* 164.08324 (±5 ppm mass window) showing consistent scan-to-scan mass accuracy as well as sufficient scans/peak for precise peak integration.
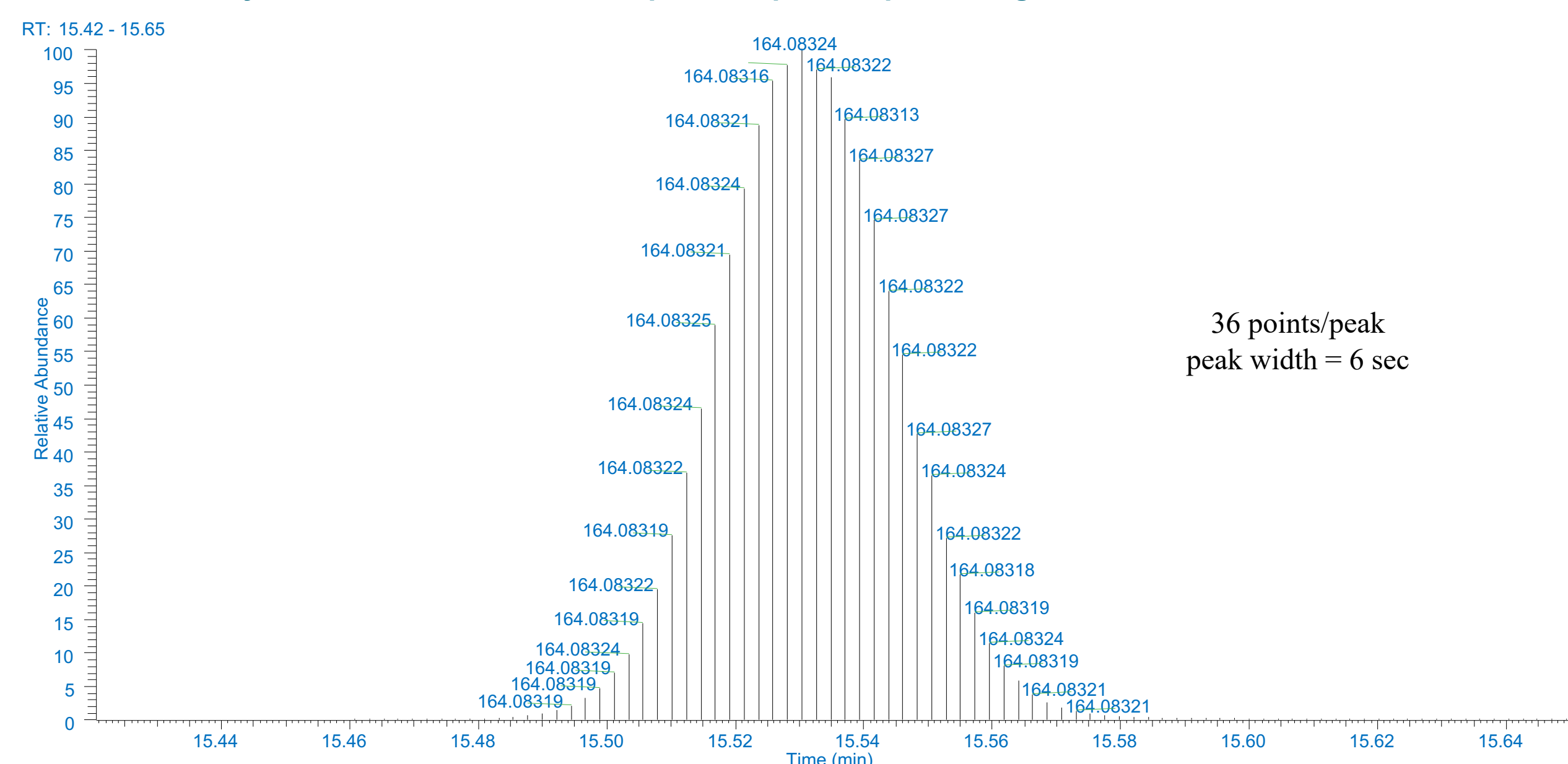

Figure 5. Principal Component Analysis (PCA) for n=6 samples suggesting significant differences among the VOC profile of pesto originating from various suppliers.


Statistical Analysis

Multivariate statistical analysis was carried out using Compound Discovered 3.1. The PCA is a well known technique that allows the identification of largest sources of variation in omics experiments such as the one performed in this study. The significant differences in the volatile profile of the samples confirmed to be strictly related to the different suppliers, probably related to production techniques used. As an example, the PCA plot for n= 9 distinct samples is shown in Figure 5. The main components responsible for group differences are: β-caryophyllene, germacrene, α-bergamotene and α-guaiene mainly originating from basil for **supplier A**, diallyl-disulfide, methyl-allyl-disulfide and diallyl-trisulfide mainly originating from garlic for **supplier B**, eugenol, eucalyptol, α-terpineol acetate, linalool acetate and humulene mainly originating from basil and pine nuts for **supplier C**.

Compound Confirmation

Further confirmation in the identification of compounds was achieved by assessing the PCI spectra. The PCI is useful for confirming the molecular ion of a chemical as only molecular ions will generate adducts formation. Unlike softer EI ionisation at lower eV energies, in PCI experiments with methane as the reagent gas three adducts are typically observed: $[M+H]^+$, $[M+C_2H_5]^+$, $[M+C_3H_5]^+$. Figure 6 shows EI and PCI spectra of eugenol with these adducts. The presence of these adducts confirm *m/z* 164.08320 as the molecular ion for eugenol.

Figure 6. Comparison between CI and EI spectra for eugenol (m/z 164.08325).
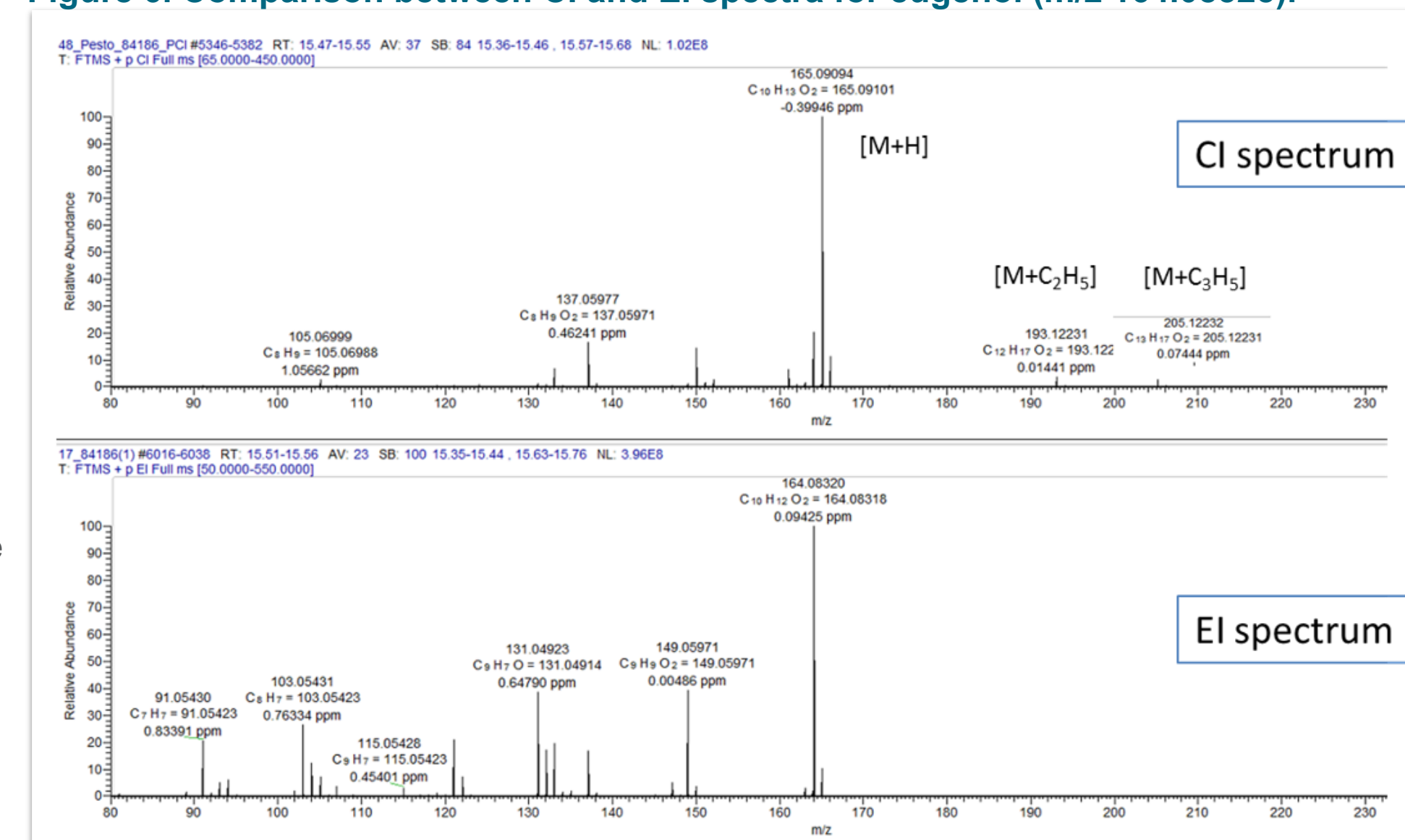
CONCLUSIONS

- The results presented in this study demonstrate that the Thermo Scientific Exactive GC hybrid quadrupole-Orbitrap mass spectrometer, in combination with easy-to-use Compound Discoverer software and SPME Arrow technology, is a powerful tool for profiling complex samples and identifying unknown peaks, critical for determining chemical components in food products originating from various vendors that employ various technological processes.

- The consistent sub-1-ppm mass accuracy, the routine resolving power at 60,000 FWHM and the wide dynamic range deliver confident detection of unknown compounds regardless of their concentration or matrix complexity.

- The EI and PCI data together with the retention index information and the total score, allow for confident compound identification.

REFERENCES

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TRADEMARKS/LICENSING

Barilla

Thermo Fisher SCIENTIFIC