Statistical Differentiation of Pesto Products Using SPME-GCxGC-TOFMS and ChromaTOF® Tile Software
Enhanced Aroma Characterization

Introduction

Pesto and its origins 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 more optimal product development and therefore higher brand awareness and customer satisfaction.

The complexity of GCxGC data can quickly become overwhelming due to a necessity of maintaining peak-tables of hundreds or thousands of analytes for each sample. To find statistically significant differences between data sets often requires time-consuming and intensive data-mining on an expert-level.

Now, *ChromaTOF* Tile, a data processing software platform developed using Prof. Synovec's Tile-based Fisher ratio approach, uses a revolutionary algorithm for the comparison of multiple GCxGC-TOFMS data in a fast and user-friendly way, while using nominalized mass traces for area calculation. Statistically significant differences can be then easily highlighted, reducing the time required to locate the differences between the sample sets.

The easy and user-friendly approach to the differentiative analysis is demonstrated on a data set of 11 pesto samples from three different producers. Samples were acquired by SPME-GCxGC-TOFMS.
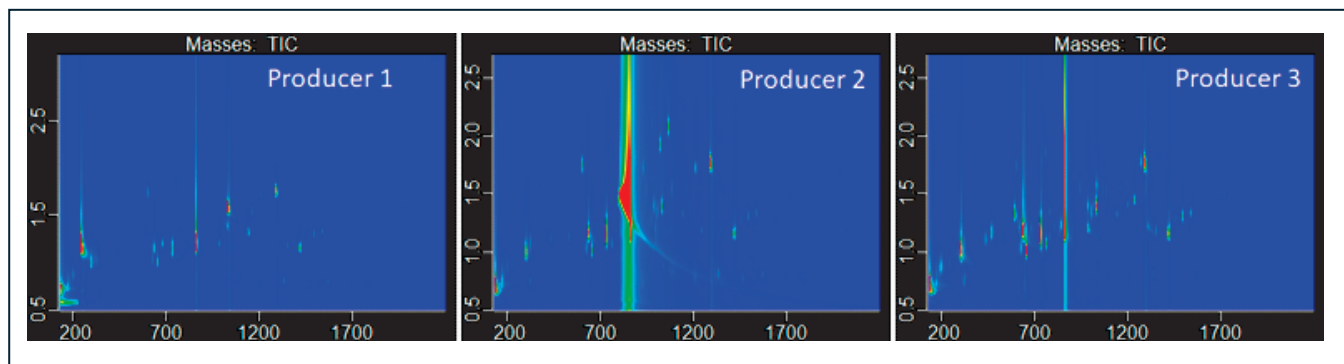

Figure 1: Representative TIC contour plots of eleven pesto samples from three different producers.

Experimental

Materials

Eleven commercially available pesto products from three different producers were analyzed in three parallel analyses to allow a robust statistical comparison of pesto aroma profiles.

n-Alkane standards (C7-C30), obtained from Merck (#49451-U) were diluted to 10 μg/mL and analyzed for calculation of linear retention indices.

Sample Extraction & Instrumental Conditions

The samples were prepared for HS-SPME by weighing pesto aliquots (1g ±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 (Merck #57329-U) which was then immediately desorbed in the GC inlet for analysis with conditions listed in Table 1. Data for an alkane standard were also acquired to calculate retention indices to refine library search results.

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 GCxGC-TOFMS contour plots of three pesto samples originated from different producers are shown in Figure 1. The aim of this case study was to find the differences in products of three producers utilizing an easy and fast differentiation software tool.

The degree of complexity in differential analysis depends on the methodology employed. A basic approach is to locate differences based on visual comparisons of contour plots using TIC and/or selected masses (e.g. 93 for terpenoids). A more advanced strategy is to utilize a statistical comparison software. The latter workflow—ideally fully automated—allows discovery of differences that aren't easily visible or would be time consuming to identify, due to low intensity and high analyte complexity across numerous samples. LECO *ChromaTOF* Tile locates the important spots by tessellating the GCxGC plots by tiles (1D, 2D RT windows). The application of Fisher-ratio (F-ratio) analysis to the supervised comparison of sample classes algorithmically reduces complex GCxGC-TOFMS data sets to find class distinguishing chemical features[2]. The area of each tile is calculated on each individual nominal mass, and the integrals are used for Fisher ratio (F-ratio) computation to discover statistically meaningful differences, as described by Synovec.[1,2]

Although multiple ways of displaying the results of differentiative analysis can be employed, one of the most used is the Principal Component Analysis (PCA) plot. It provides clustering of samples based on their similarity. Figure 2 shows the PCA obtained for this case study.
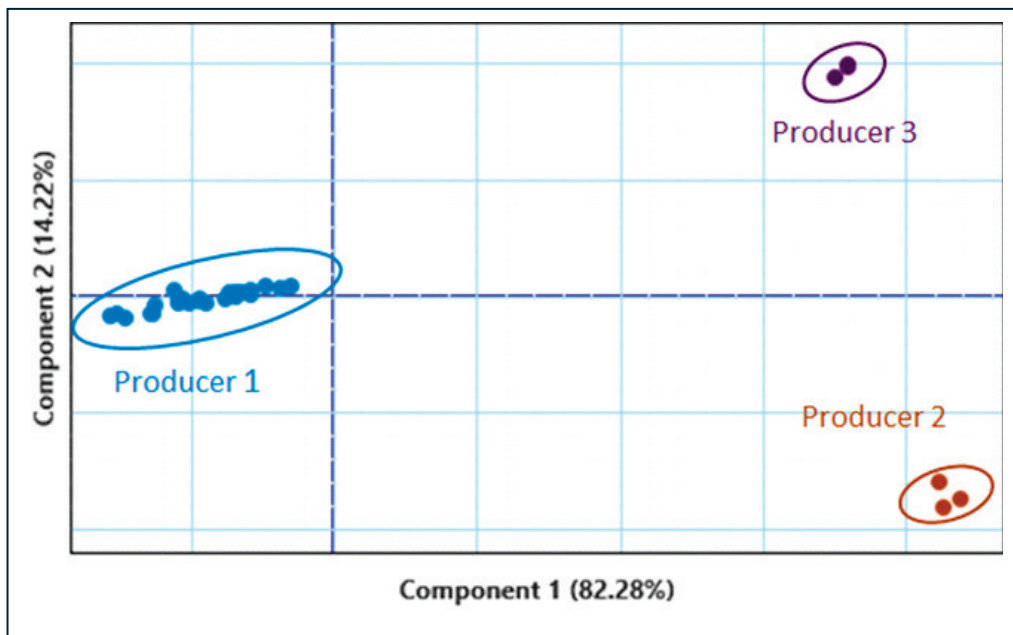
Figure 2: PCA plot with great separation of sample classes.

The contribution of each feature to sample clusterization is summarized via a loadings plot as shown in Figure 3, where the most distinguishing components are highlighted, and their identification is performed by comparison of background subtracted mass spectra within *ChromaTOF* Tile using NIST MS libraries and retention indices calculations. The following compounds were found to be most distinguishing components:

(i) 1,2-Propanediol. This feature is shown in detail in Figure 4. It was observed mainly in sample class Producer 1, while in samples from Producer 2 and 3 was not present.

(ii) In Figure 5 butanoic acid prominence is revealed as a statistically meaningful difference in producer 3's sample set. Similarly, 7-Hydroxyocta-2,4-dienoic acid was exclusively found in producer 2's products. (Figure 6). Besides these two features, other short-chained organic acids (e.g. hexanoic- and propanedioic-) were also recognized as other markers.

(iii) Estragole was mostly present in Producer 1's samples and slightly in Producer 3's, while in samples from Producer 2 was not found at all, as indicated in Figure 7.
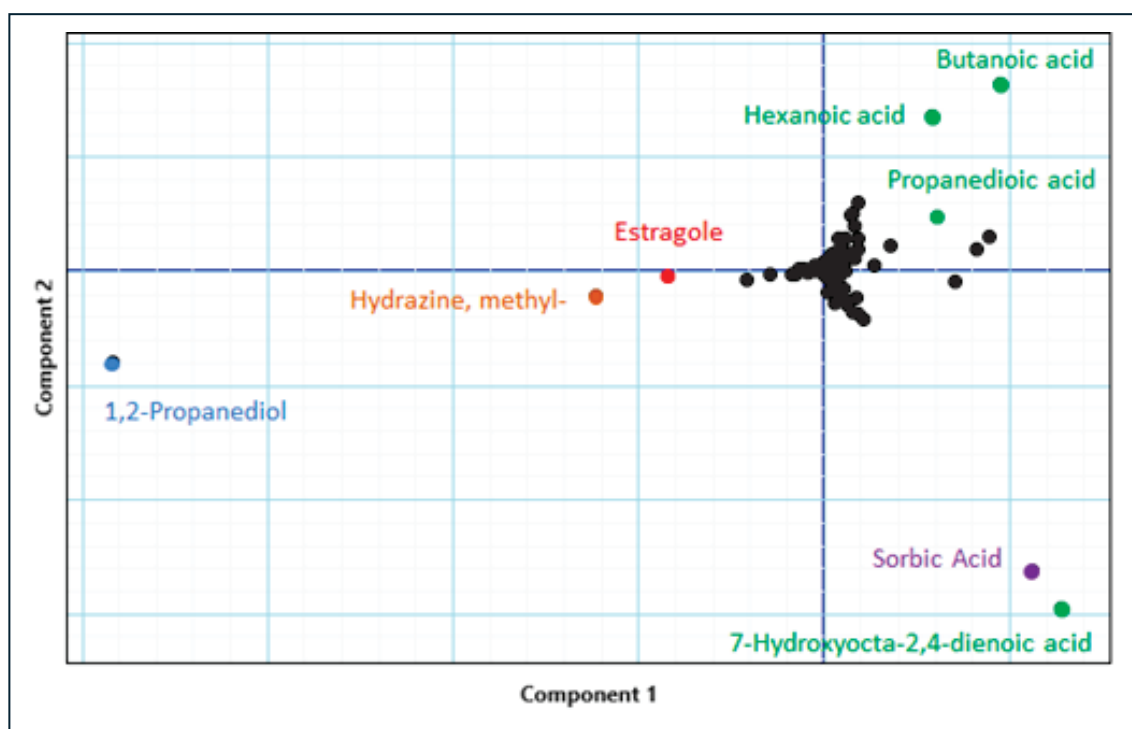

Figure 3: Loading plot of above mentioned PCA plot with most marked distinguishing components identified as 1,2-Propanediol, butanoic acid, 7-Hydroxyocta-2,4-dienoic acid, hexanoic acid, propanedioic acid, Estragole, Methylhydrazine and Sorbic acid.
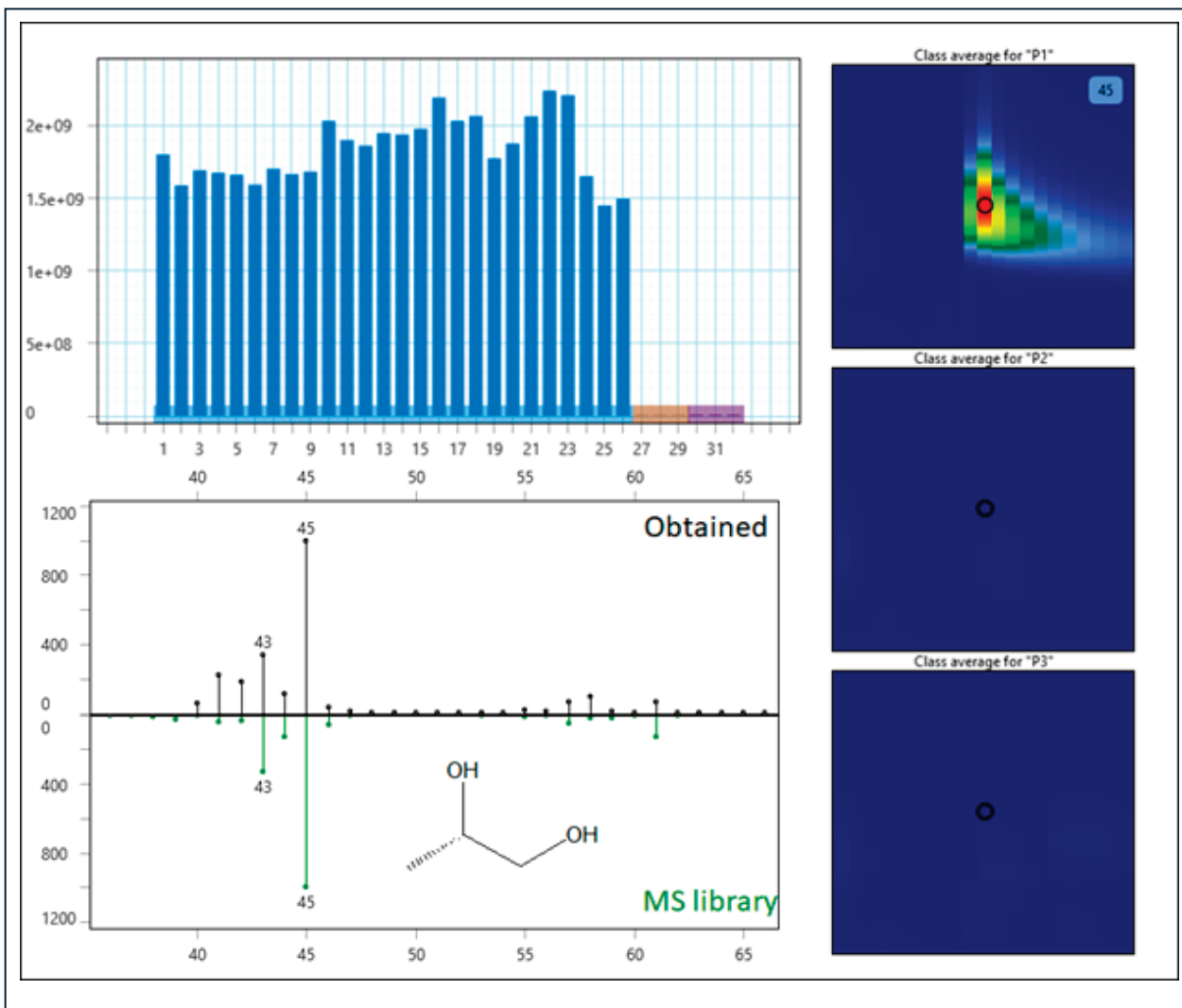
Figure 4: Comparison of area bars along with an average contour plot overview for 1,2-Propanediol. Similarity match of spectra with background removal is as high as 799/1000. RI calculated value (732) was compared with RI provided by NIST (732).
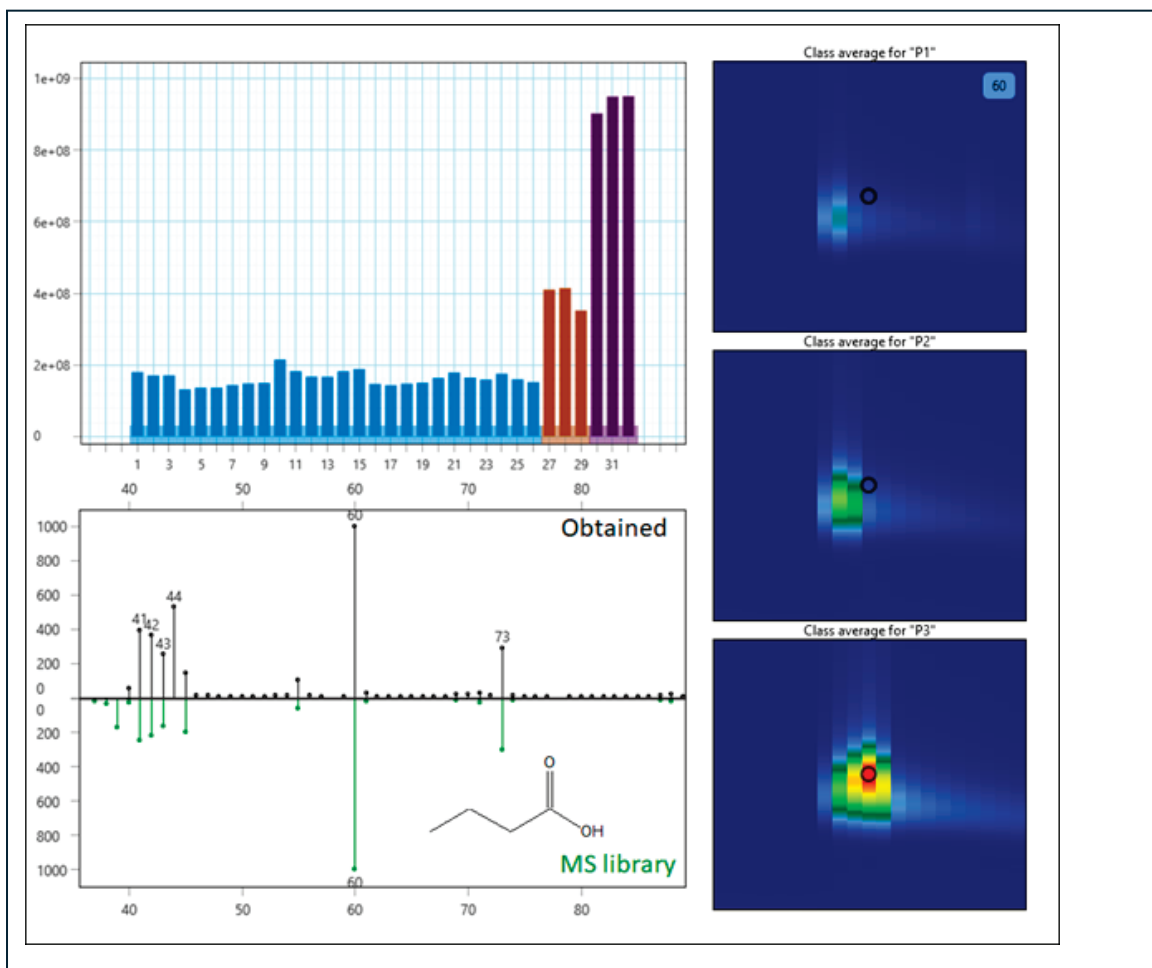
Figure 5: Details of a feature identified as Butanoic acid with library match 960/1000, RI 789 (802 in NIST). The feature profile and sample area bars are compared here at m/z 60.
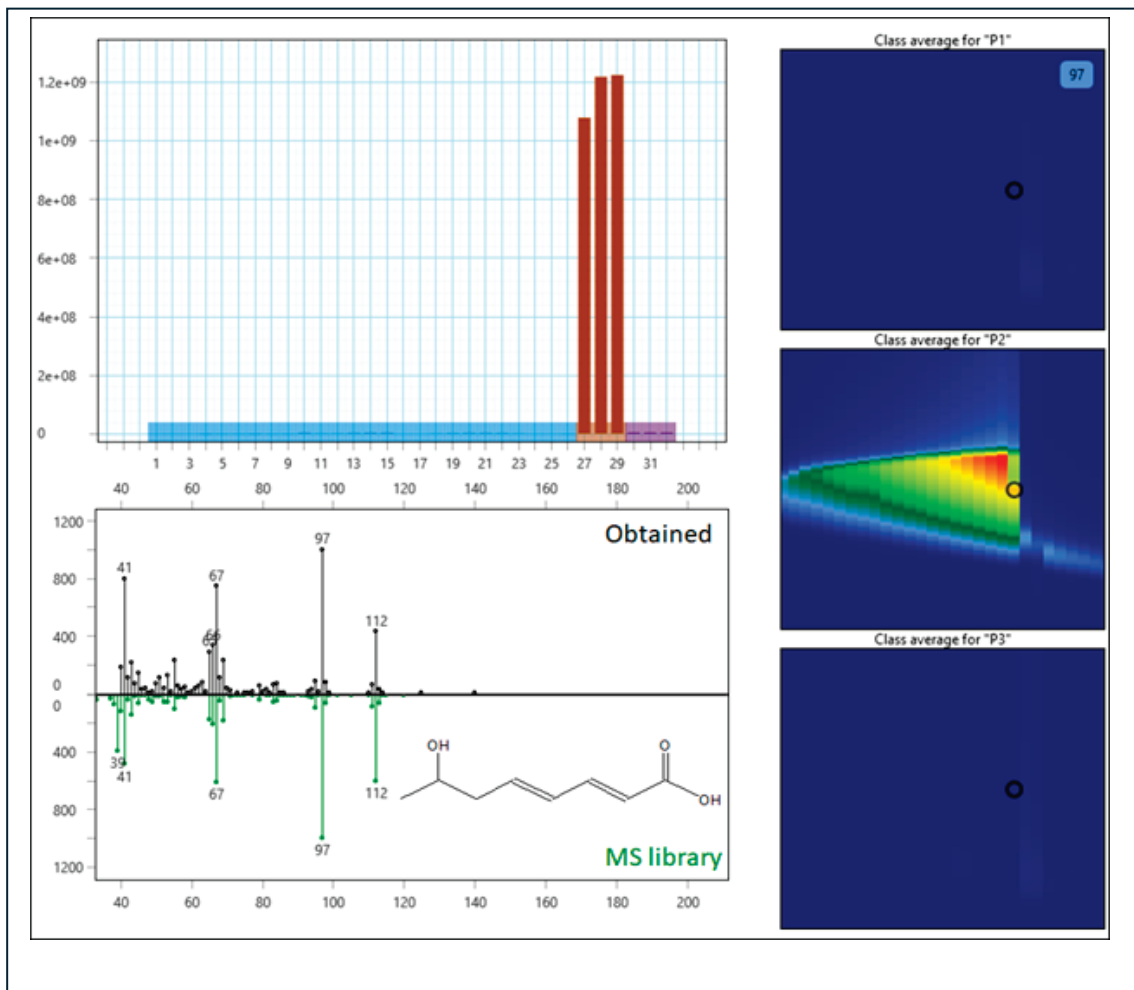
Figure 6: Average contour plots of details of 7-Hydroxyocta-2,4-dienoic acid (library match 854/1000. The feature was observed in only Producer 2's set, as summarized in bar plot.
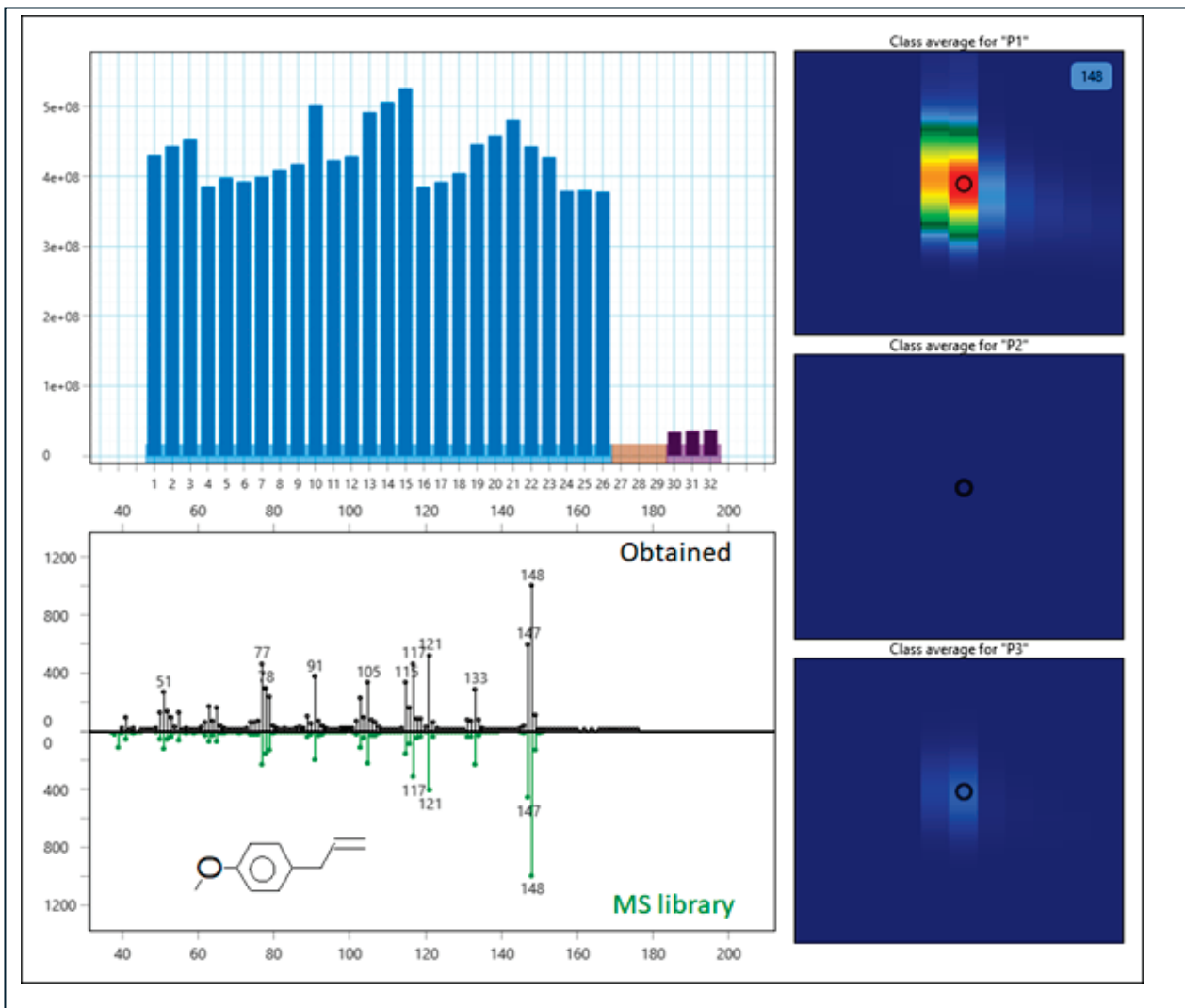
Figure 7: Comparison of area bars along with a contour plot overview indicates that the Estragole feature (similarity 923/1000, measured RI 1199/1196 by NIST) was found at high intensities in Producer 1's samples, while in Producer 3's was found at small levels and was completely missing in Producer 2's.

Conclusion

The capability of *ChromaTOF* Tile software to facilitate a fast class differentiation in non-targeted approaches for multiple samples was demonstrated in this application note. The features that are responsible for statistically meaningful samples' clustering were easily found and identified, thus highlighting key ingredient differences which could then, for example, aid product development processes in conjunction with sensory and consumer test data. In general, the utilization of this differential analysis tool allows enriched information and trends and patterns to be leveraged quickly in complex sample sets obtained by GCxGC-TOFMS. Therefore more time can be spent focusing on meaningful results, rather than laborious data mining.

References

[1]L.C. Marney, W.C. Siegler, B. A.Parsons, J. C.Hoggard, B.W. Wright, R.E. Synovec, **Talanta,** 115 (2013) 887-895, http://dx.doi.org/10.1016/j.talanta.2013.06.038

[2]B. A. Parsons,L.C. Marney, W.C. Siegler, J.C. Hoggard, B.W. Wright, R.E. Synovec, *Anal. Chem* 87 (2015) 3812-3819, DOI: 10.1021/ac504472s

Authors

Sebastiano Panto[*], Michele Suman[**], Tomas Kovalczuk[*], Nick Jones[*]

[*]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 203-821-641, where we evaluated 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.