

Instrument: Pegasus[®] BTX 4D and ChromaTOF[®] Sync 2D

Enhanced GCxGC Data Processing by ChromaTOF[®] Sync 2D for Traditional Belgium Beer Aroma Analysis and Comparison

LECO Corporation; Saint Joseph, Michigan USA

Key Words: ChromaTOF Sync 2D; Aroma Profiling; Beer Analysis; SPME; GCxGC; TOFMS



Introduction

Aroma plays a crucial role in the sensory properties of food and beverages, making the analysis of volatile compounds essential for understanding and optimizing industrial processes like beer brewing.^[1,2] Each beer and brand have a unique aroma profile influenced by its ingredients and brewing process. The volatile profile of beer provides valuable insights into raw materials, fermentation, maturation, and potential defects. Among these compounds, fatty acid esters are key contributors to beer aroma due to their low odor threshold, along with terpenoids, aldehydes, and ketones.^[3,4] Aroma analysis is essential for understanding the sensory characteristics of beer and often requires advanced analytical techniques and efficient data processing workflows. Comprehensive two-dimensional gas chromatography (GCxGC) provides high-quality data, but effective interpretation relies on robust software tools. In this study, *ChromaTOF Sync 2D* was applied to enhance GCxGC data processing for the aroma analysis of beers from Val-Dieu, a traditional Belgian abbey brewery with a rich history of fermentation expertise.^[5]

Experimental

In total, seven Belgian Trappist beers kindly provided by Val-Dieu (see Table 1) were analyzed using headspace solid-phase microextraction (HS-SPME). After degassing, 1 g NaCl was added to 10 mL of sample in a 20 mL HS vial. After an incubation time of 1 min at 40 °C, a DVB/CAR/PDMS SPME fiber was exposed for 15 min and then desorbed on a split/splitless injector. Table 2 summarizes the parameters applied for the injection conditions and the GCxGC-TOFMS analysis. Samples were injected in triplicate, and an n-Alkane standard (C7–C30) was also analyzed for calculation of linear retention indices (RI).

Table 1: Analyzed Belgium Trappist Beers from the Abbey Val-Dieu.

Nr.	Name	Style
1	Blonde	Belgian Pale Ale
2	Brune	Belgian Dubbel
3	Fruitée	Fruit Beer
4	Grand Cru	Quadruple
5	Hop	Belgian Ale
6	Noël	Christmas Ale
7	Triple	Tripel



ChromaTOF[®]
SYNC 2D

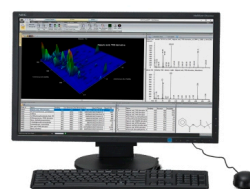


Table 2: GCxGC-TOFMS analysis parameters.

GC Parameters	Agilent 8890
Injector	split (1:50), 250 °C
Carrier Gas	Helium, 1.40 mL/min, constant flow
Columns	Rxi-5 ms 30 m x 0.25 mm x 0.25 μ m (Restek) Rxi-17 Sil ms 0.6 m x 0.15 mm x 0.15 μ m (Restek)
Oven Program	50 °C (3 min), 5 °C/min to 240, 10 °C/min to 270 °C (1 min)
Secondary Oven Temp	+5 °C (relative to the GC oven temperature)
Modulator Temp	+15 °C (relative to the secondary oven temperature)
Modulation Period	2.5 s
Transfer Line	300 °C
TOFMS	LECO Pegasus BTX 4D LN2
Ion Source Temp	250 °C
Mass Range	40–400 m/z
Acquisition Rate	100 spectra/s

Results and Discussion

Understanding the variation in aroma components is essential for comprehending the quality of beer and other beverages. Non-targeted analytical approaches, such as gas chromatography with mass spectrometry (GC-MS), can offer great insights regarding the aroma composition. However, pairing an additional complementary separation, such as that obtained by comprehensive two-dimensional gas chromatography (GCxGC) with time-of-flight mass spectrometry (TOFMS), provides ultimate resolving power and improved confidence in identifications to build the basis for state-of-the-art aroma profiling.

Thus, the use of GCxGC-TOFMS for the analysis of beers provides improved understanding of the aroma composition. GCxGC typically results in better chromatographic separation for many analytes that would be coeluting in a single chromatographic dimension separation. One single analytical run already results in a great number of detected analytes, many of them with important odor characteristics. In order to investigate these analytes across larger data sets, including replicates, one needs to have sophisticated data analysis tools to handle and streamline data processing.

Figure 1 demonstrates the power of GCxGC on the example of β -myrcene (CAS: 123-35-3) and 2-octanone (CAS: 111-13-7). In this case, the two compounds have the same experimental retention time in the first dimension (RT₁) and the corresponding library retention indices (RI_{lib}) for both compounds are identical. However, these two analytes were well separated in the second dimension allowing for a straightforward identification using the mass spectral information. Both compounds are odor active and contribute to the aroma profile of the beer.

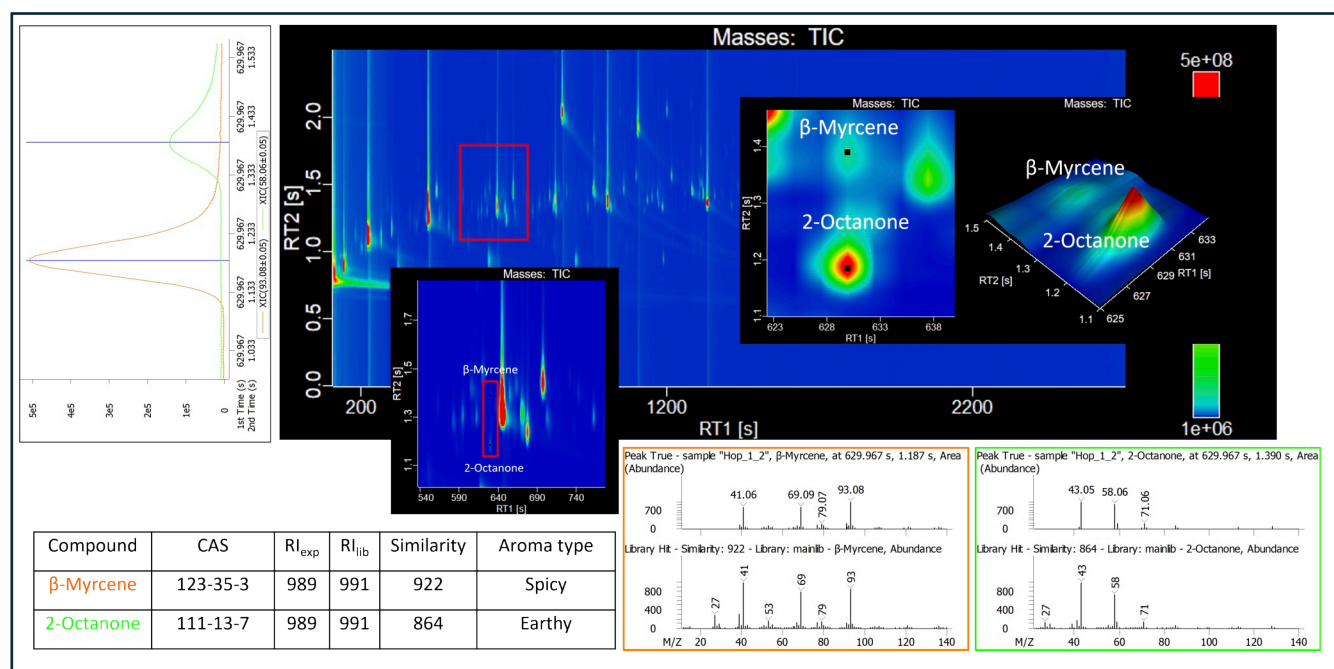


Figure 1: GCxGC separation of an HS beer sample with a zoomed-in area of two compounds having similar RT₁. Here, β -myrcene and 2-octanone were chromatographically separated in the second dimension. Thanks to this improved separation the analytes could be identified easily.

The data processing workflow aimed to compare the different Trappist beers and investigate analyte differences and similarities. However, the amount of data generated by multiple GCxGC runs can be overwhelming and analytical chemists encounter persistent challenges when performing these types of non-targeted analyses of multiple data files. *ChromaTOF Sync 2D* is an advanced peak finding and alignment tool delivering comprehensive insights for complex GCxGC-data sets and was therefore chosen as a means of achieving this goal. As an output, chemical information from each sample is compiled in the so-called “Aligned Results Table” (ART), forming the foundation for further data investigation. Several options for visualization such as heatmaps and charts allow for exploring the trends of individual analytes or groups of compounds.

The ART in Figure 2 compiles a lot of information and the display can be tailored according to the user’s needs. In Figure 2 on the right side, the heat map is enabled, helping with the visualization of trends. Each column in the table corresponds to a single sample. Columns 1-3 are replicates of the Blonde beer, 4-6 are Brune, 7-9 are Fruitée, 10-12 are Grand Cru, 13-15 are Hop, 16-18 are Noël, and 19-21 are Triple. The color scale indicates relative intensity where red is higher and blue is lower, so color trends across each row indicate that analyte’s relative amount in the various beer samples.

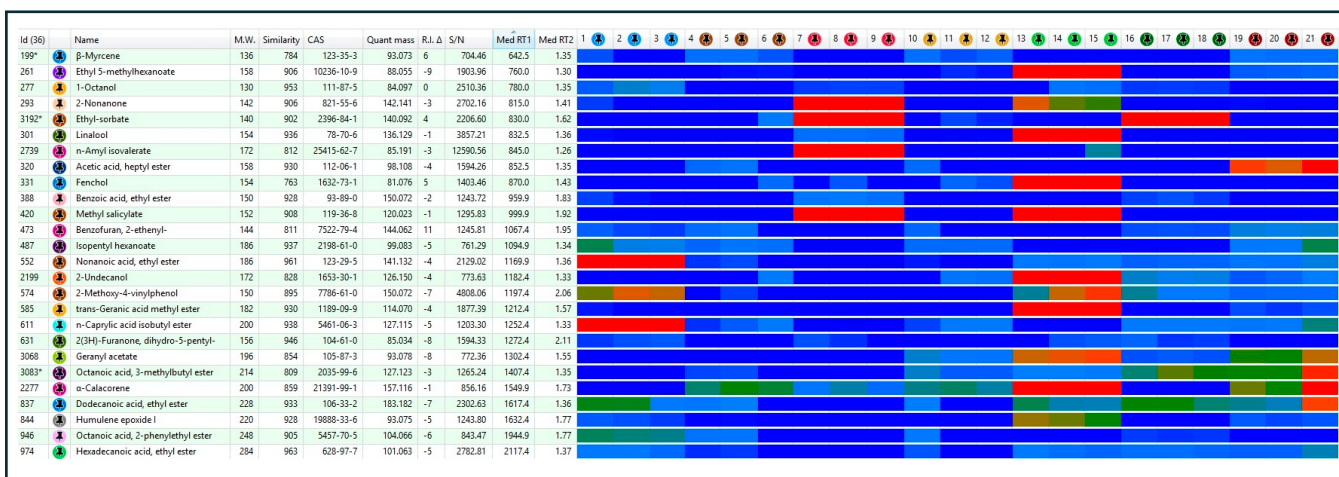


Figure 2: Peak identification metrics (similarity score, RI information) and relative trends (heat map color scale) for representative analytes from various compounds.

For instance, geranyl acetate (CAS: 105-87-3), having a citrus-type odor, is particularly high in two of the seven Trappist beers, whereas the other beers display a medium or relatively low level. This can be observed in the heat map in Figure 2 and also in the detailed information shown in Figure 3. Another example is ethyl sorbate (CAS: 2396-84-1), with green being a fruity odor, shown in Figure 4. This component is highly present in the fruity beer, and was also detected in the “Christmas-style” beer, but is totally absent in the others. As demonstrated with these two examples (see Figure 3 and Figure 4), features can be explored individually, and their relative concentrations can be displayed using a bar chart, for example.

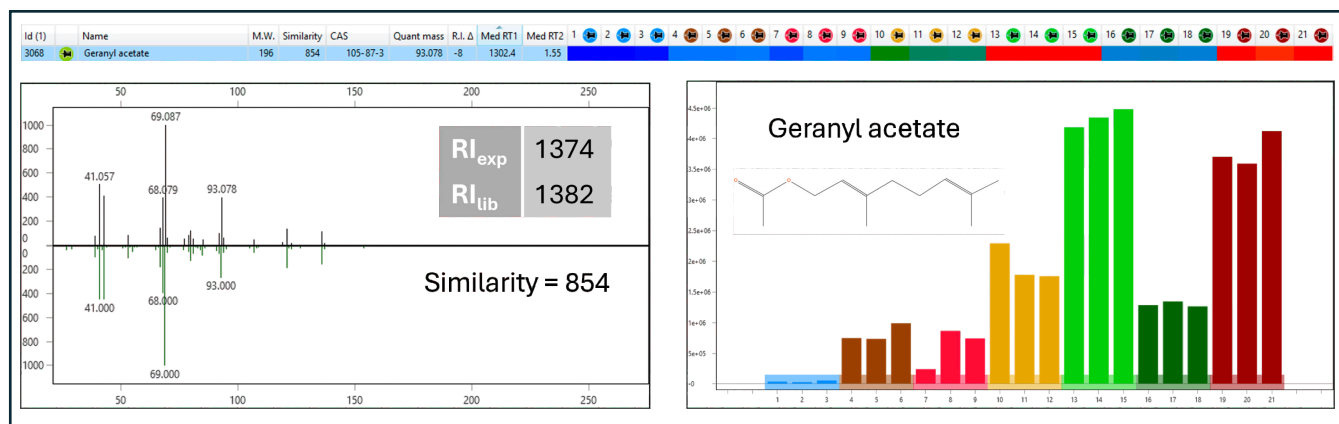


Figure 3: Relative trend of geranyl acetate (CAS: 105-87-3) identified through mass spectral and RI matching.

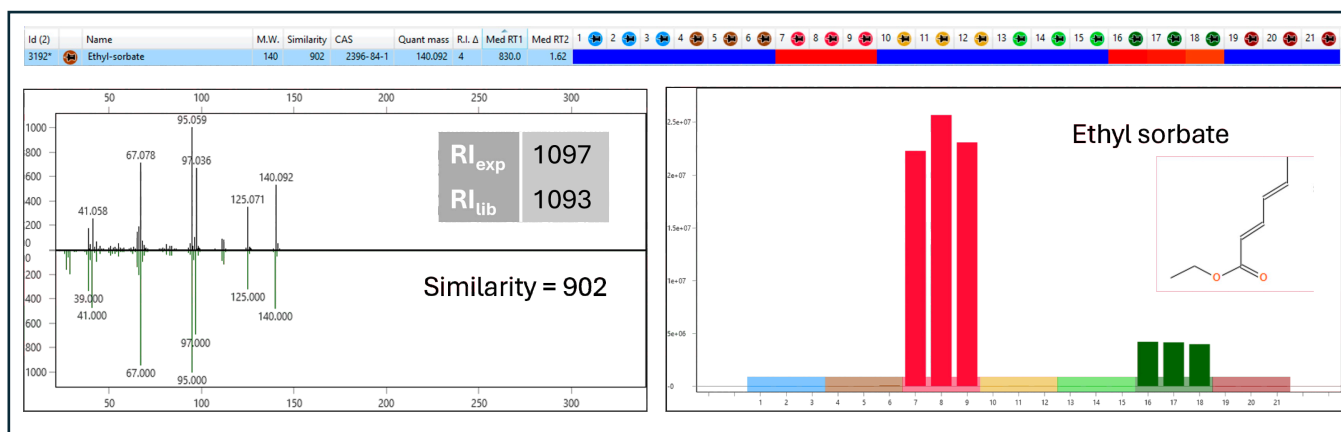


Figure 4: Relative trend of ethyl sorbate (CAS: 2396-84-1) identified through mass spectral and RI matching.

Furthermore, class comparison based on ANOVA (Analysis of Variance) to determine statistical significance of features between sample groups or classes can help to highlight compounds of interest that distinguish the samples. To gain an overall picture, Principal Component Analysis (PCA), which is commonly employed for the reduction of high dimensional data, can be conducted to reveal clustering based on chemical similarities and differences. Figure 5 shows the scores plot as a result of the PCA run on the seven analyzed Trappist beers. The distinct grouping of the sample reflects the ingredients and specific recipes for the different beers.

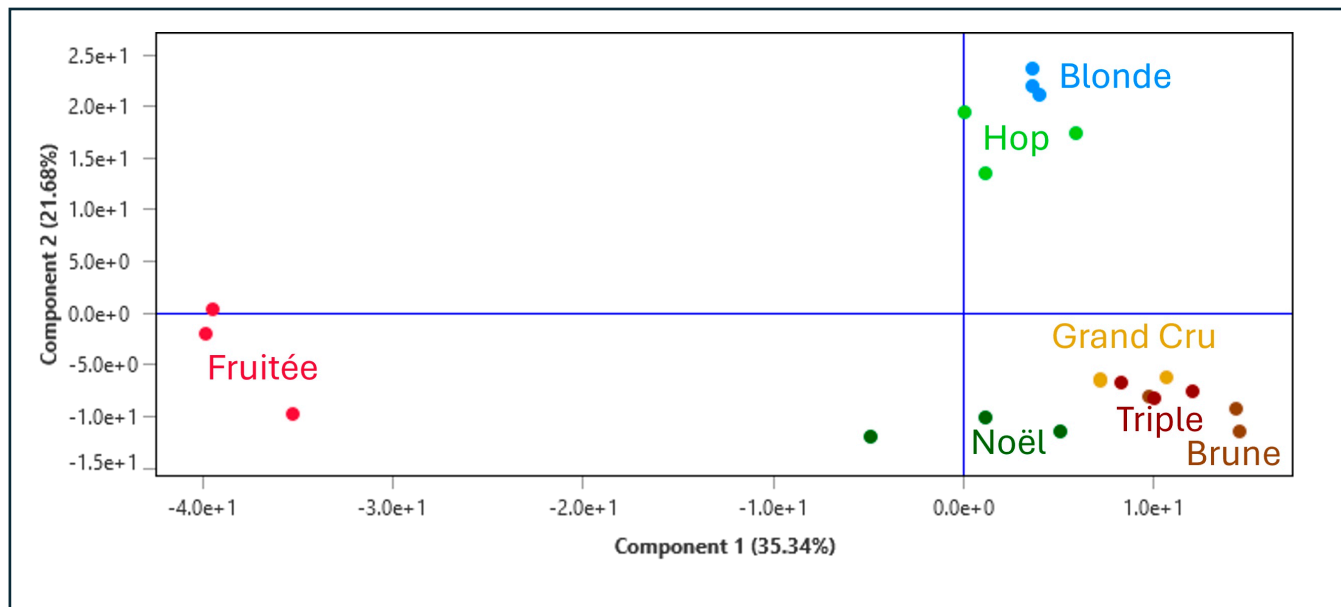


Figure 5: Principal Component Analysis (PCA) Scores plot shows three distinct main clusters for the seven Trappist Beers. Furthest apart on the left side is the fruity beer; on the top right side, the blond and the hop beer are relatively close; and on the bottom right side the four special beers are grouped together.

Conclusion

The analysis of traditional Belgian Trappist beers using GCxGC-TOFMS combined with *ChromaTOF* Sync 2D provided deep insights into their complex aroma profiles. The enhanced chromatographic separation enabled the identification of key volatile compounds, while advanced data processing tools facilitated the comparison of different beers, revealing trends and unique characteristics. The use of statistical methods such as ANOVA and PCA further highlighted significant variations between beer samples, demonstrating the effectiveness of this approach for quality control and product development. This study underlines the importance of comprehensive analytical techniques in combination with state-of-the-art software tools to refine brewing processes and preserve the distinctive sensory attributes of traditional beers.

Acknowledgement

Maxime Pinckaers, Val-Dieu, Belgium

OBiAChem, University of Liège, Liège, Belgium

References

- ^[1]Shellie, R.A. et al. (2009). Varietal characterization of hop (*Humulus lupulus* L.) by GC–MS analysis of hop cone extracts. *Journal of Separation Science*. <https://doi.org/10.1002/jssc.200900422>.
- ^[2]Ceola, D. et al. (2019). Headspace-solid phase microextraction and GC-MS followed by multivariate data analysis to study the effect of hop processing type and dry hopping time on the aromatic profile of top-fermented beers. *SEPARATION SCIENCE PLUS*. <https://doi.org/10.1002/sscp.201900012>.
- ^[3]Olaniran, A.O. et al. (2017). Flavour-active volatile compounds in beer: production, regulation and control. *Journal of the Institute of Brewing*. <https://doi.org/10.1002/jib.389>.
- ^[4]Holt, S. et al. (2019). The molecular biology of fruity and floral aromas in beer and other alcoholic beverages. *FEMS Microbiology Reviews*. <https://doi.org/10.1093/femsre/fuy041>.
- ^[5]<https://www.abbaye-du-val-dieu.be/en/>.