Application Note

Instrument: Pegasus® BT 4D

Differentiation of Regular and Barrel-Aged Maple Syrups with ChromaTOF® Tile™

Key Words: GCxGC, TOFMS, Differential Analysis, *ChromaTOF Tile*, Fisher Ratio, Class Comparison, Syrup, Barrel-Aging

Introduction

Bourbon barrel-aging is a trend that has spread through a wide range of food and beverage markets. With this process, a charred oak barrel that had previously been used to age bourbon is repurposed for aging a different food or beverage product, typically for several months. During this process the food or beverage can absorb flavors from the oak barrels and the residual bourbon in the barrels, and flavors originally present in the food or beverage can also infuse into the barrel and/or undergo reactions with time. This process tends to add both complexity of flavor and value to the products and has been used for beers, liquors, wines, sauces, syrups, coffees, and teas. In this application note, we explore specific chemical differences between a maple syrup that was bourbon barrel-aged and a maple syrup that was not. To achieve this analytical goal, the chemical components in the sample were separated with two-dimensional gas chromatography (GCxGC), which couples two columns with complementary stationary phases to increase the peak capacity relative to using either stationary phase alone as a single dimension separation. This chromatographically isolates more individual analytes in these complex samples. Pairing this powerful separation with time-of-flight mass spectrometry (TOFMS) allows for the chemical identification of these isolated compounds based on matching to spectral databases. GCxGC-TOFMS creates rich data that can then be mined with data analysis software tools to help reveal the meaningful information and answer the analytical questions. In this case, *ChromaTOF Tile* was used to rapidly compare the raw data across the sample set to find class-differentiating features, the specific chemicals that distinguish the bourbon barrel-aged maple syrup from the regular maple syrup.

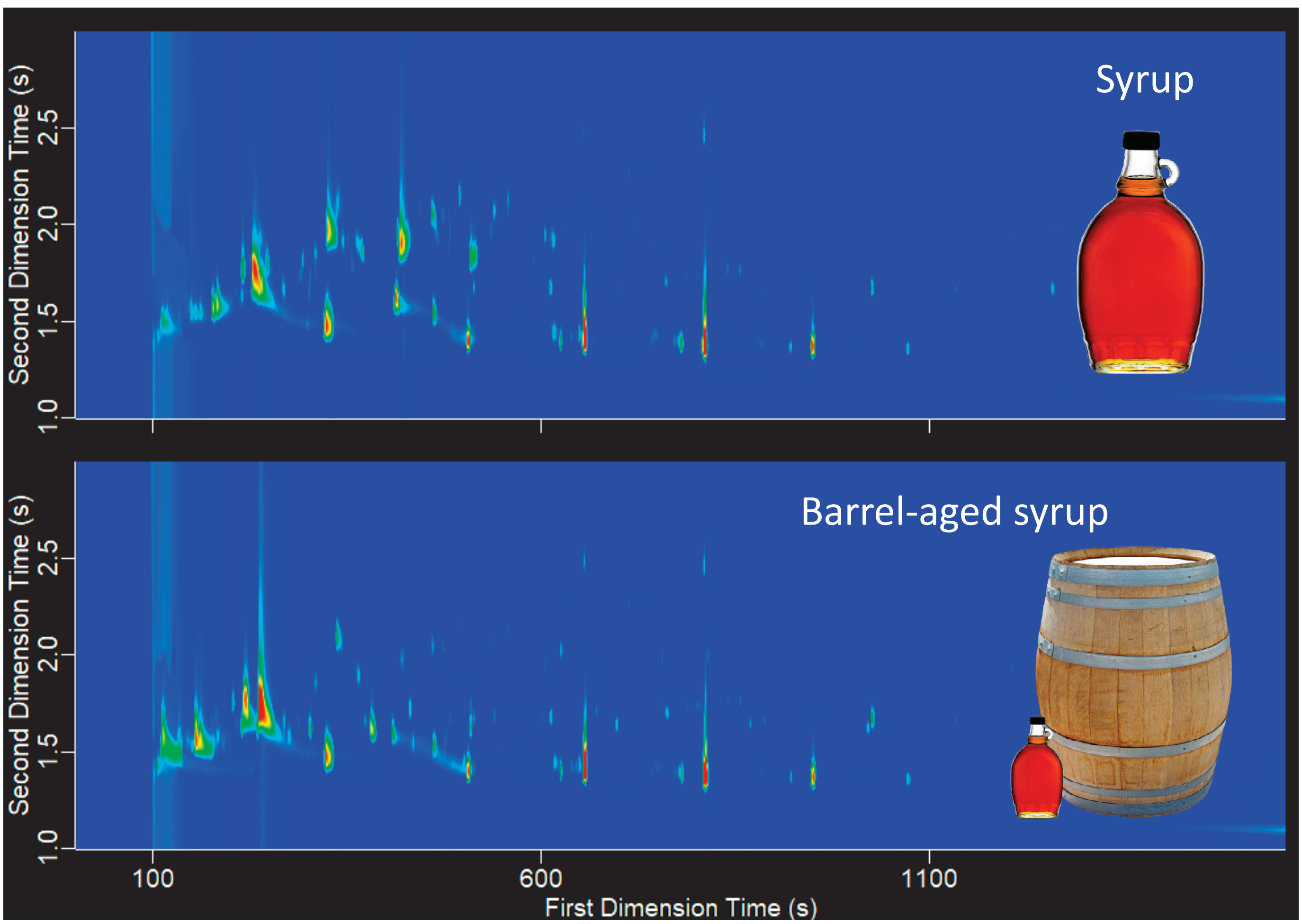

Figure 1. Representative chromatograms of bourbon barrel-aged and regular maple syrup.

Experimental

Two types of syrup samples, one regular and one bourbon barrel-aged, were analyzed with HS-SPME and GCxGC-TOFMS. The samples were diluted to approximately 50% (1 g syrup per 1 mL water) in distilled water. A 2 mL aliquot of each diluted syrup was then added to a 20 mL HS-SPME vial. The samples were incubated at 40 °C for 5 min and extracted for 10 min at the same temperature with a tri-phase SPME fiber (PDMS/DVB/Carboxen, Supelco). GCxGC-TOFMS conditions are listed in Table 1. Data were acquired in quadruplet for each type of syrup and the SPME fiber was conditioned for 5 min at 250 °C between injections. Data for an alkane standard was also collected with the same methods in order to perform Retention Index (RI) calculations. Sample acquisition was automated and controlled with LECO's *ChromaTOF* software and data analysis to determine and identify the class-differentiating features was performed with *ChromaTOF Tile*.

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

Auto Sampler	**LECO L-PAL 3 Autosampler**
Injection	2 min desorption in GC inlet, splitless
Gas Chromatograph	**LECO GCxGC QuadJet™ Thermal Modulator**
Inlet	250 °C
Carrier Gas	He @ 1.4 mL/min, corrected constant flow
Columns	HP-5 MS, 30 m x 0.25 mm i.d. x 0.25 µm coating Rxi-17SilMS, 0.45 m x 0.25 mm x 0.25 µm coating
Temperature Program	Hold 2 min at 40 °C, ramp 10 °C/min to 280 °C Secondary oven: +20 °C relative to primary oven
Modulation	2 s with temperature maintained +15 °C relative to 2nd oven
Transfer Line	300 °C
Mass Spectrometer	**LECO *Pegasus* BT**
Ion Source Temperature	250 °C
Mass Range	35-550 m/z
Acquisition Rate	200 spectra/s

Results and Discussion

Representative contour plots of the regular and bourbon barrel-aged syrups are shown in Figure 1. There are some similarities and some distinct differences between the samples. The primary objective of the analysis was to determine and understand these chemical differences because they may relate to the barrel-aging process. Some of the differences are apparent in the TIC and these would likely be determined through visual comparison of the chromatograms. Other differences, however, may be more difficult to determine without the use of comparative software tools. Features that are at low levels may not be visible in the TIC while other distinguishing features may be obscured by coelution and sample complexity. LECO's *ChromaTOF Tile* can help draw these differences out of the data by rapidly locating spots in the chromatogram (retention windows and specific m/z) that distinguish the sample groups in a statistically meaningful way. This comparison is done on each m/z in the raw data so it uncovers features that may not be obvious visually and leads to a better understanding of the sample groups and their differences.

One of the most-distinguishing features between the types of syrups was pyrazine, shown in Figure 2. Pyrazine was only observed in the regular syrup (samples 5-8, dark brown bars) and not detected in the barrel-aged syrup (samples 1-4, light brown bars). *ChromaTOF Tile* determined the location in the data that this feature appeared and then returned a tentative identification using the spectral and retention information. The observed spectral data is shown on top and the library match, with a similarity score of 904, is shown inverted below on the left panel of Figure 2. The tentative identification is also supported with retention index with an observed RI value of 745 compared to the library RI value of 736. Pyrazine is described with "nutty" aroma descriptors and this is likely an important difference that also has implications in the aroma profile.

ChromaTOF Tile was crucial for uncovering this important difference in the samples. A review of the TIC in this retention area, shown in Figure 3, suggests that pyrazine may have been difficult to determine if only a visual comparison of the TIC was performed. The averaged TIC chromatogram for the barrel-aged syrup samples (top) is compared to the averaged TIC chromatogram of the regular syrup samples (bottom) in the left panel of Figure 3. The black circle marker indicates the retention time for pyrazine and the difference is not necessarily obvious. What does stand out in the TIC is a difference for a feature that is actually higher in the barrel-aged syrup. This feature, that obscures pyrazine in the TIC, can also be noted in the XIC for m/z 55, shown in the right panel. And, pyrazine, while not clear in the TIC, can be noted with XICs for m/z 80, shown in the middle panel. The second difference likely would have been noted in the TIC, but by exploring all m/z with *ChromaTOF Tile*, both of these class-distinguishing features were determined.

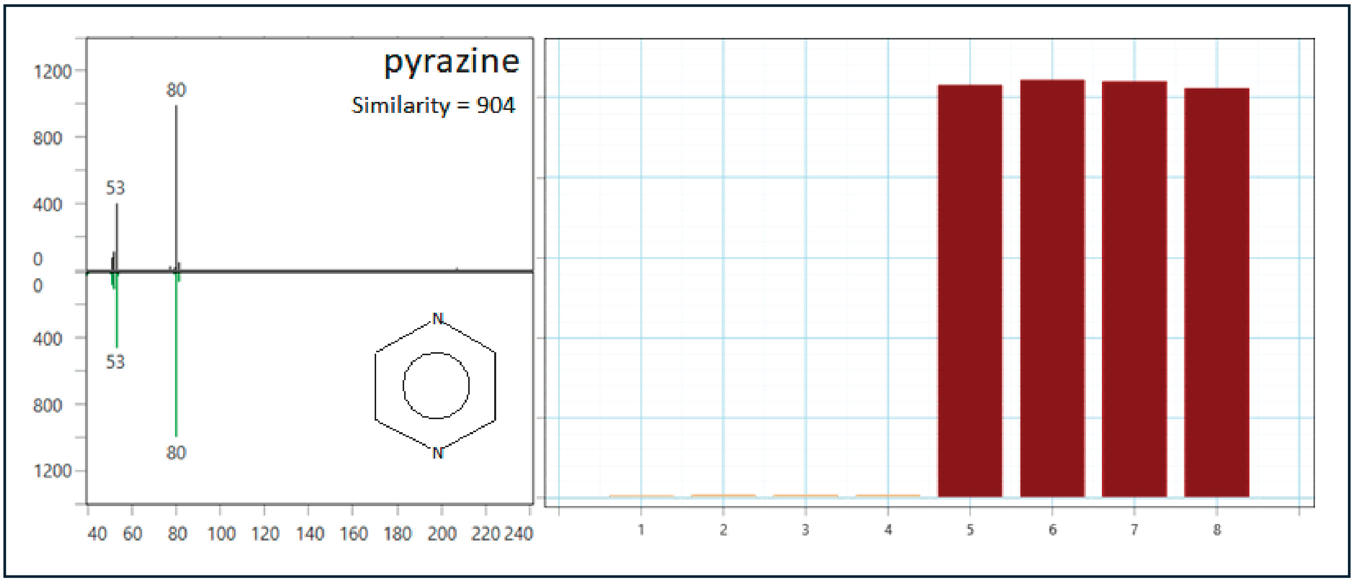

Figure 2. Pyrazine was determined as a class-distinguishing feature through ChromaTOF Tile. It was identified through spectral and RI matching and is observed at higher levels in the regular syrup (samples 5-8, dark brown).

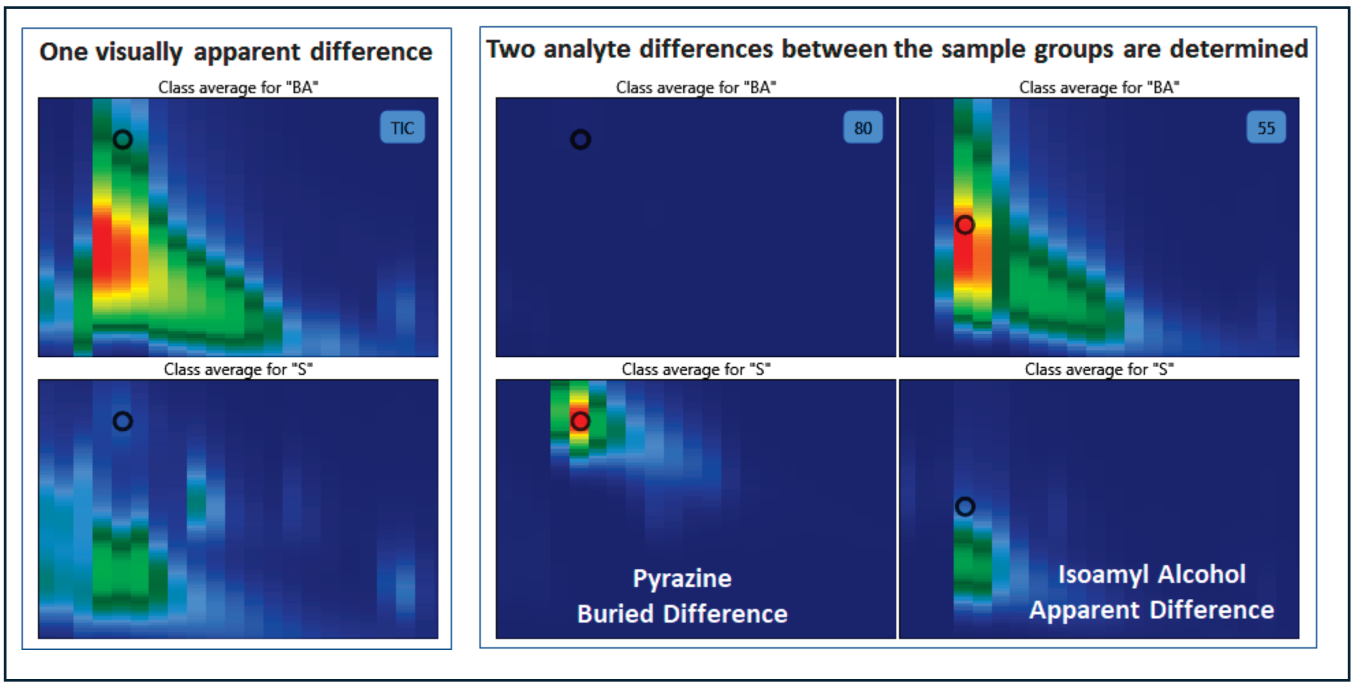

Figure 3. The average chromatogram for the barrel-aged syrup (top) and regular syrup (bottom) are shown for the TIC (left), m/z 80 (middle), and m/z 55 (right) in the chromatographic window around pyrazine.

Details for the second feature shown in Figure 3, are shown in Figure 4. This feature was determined to be isoamyl alcohol and was observed at higher levels in the barrel-aged syrup (samples 1-4, light brown) compared to the regular syrup (samples 5-8, dark brown). *ChromaTOF Tile* determined this difference and the identification was assigned from both the spectral and retention information. The observed spectral data is shown on the top spectrum and the library match, with a similarity score of 917, is shown inverted below. The tentative identification is also supported with retention index with an observed RI value of 742 compared to the library RI value of 736. Isoamyl alcohol is described with "fermented" and "whisky" aroma descriptors and the higher levels in the barrel-aged sample are relevant to the differences in the aroma profile.

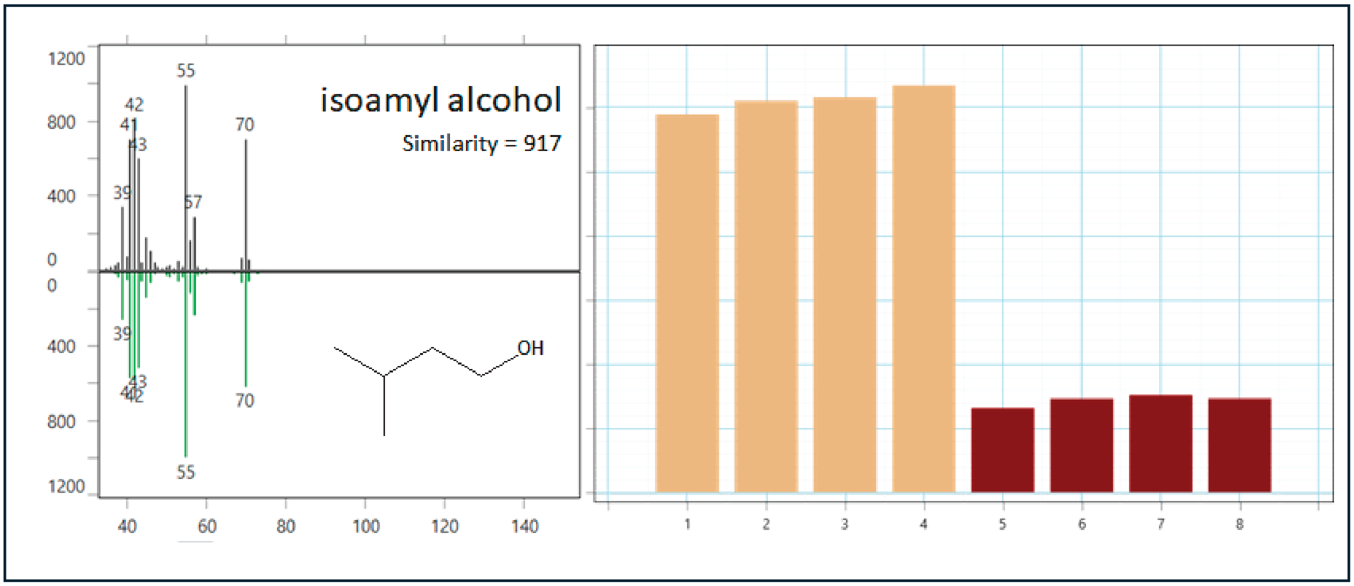

Figure 4. Isoamyl alcohol was determined as a class-distinguishing feature through ChromaTOF Tile. It was identified through spectral and RI matching and is observed at higher levels in the barrel-aged syrup.

In addition to the two features shown in Figures 2-4, *ChromaTOF Tile* returned information on many of other features in the chromatogram that distinguished the samples. The relative trends between the samples for these features and their tentative identifications were determined with the software. The overall trends were then summarized and characterized with PCA in *ChromaTOF Tile*. One hundred and thirty-nine features that were determined to distinguish the samples and that were also tentatively identified (by similarity score and RI matching, when available) were submitted as variables for PCA. As only chemical features that distinguish the groups were selected as variables, the scores plot is expected to show distinct clusters by group, as shown in Figure 5. Indeed, almost all of the variation is captured in the first principal component (PC1).

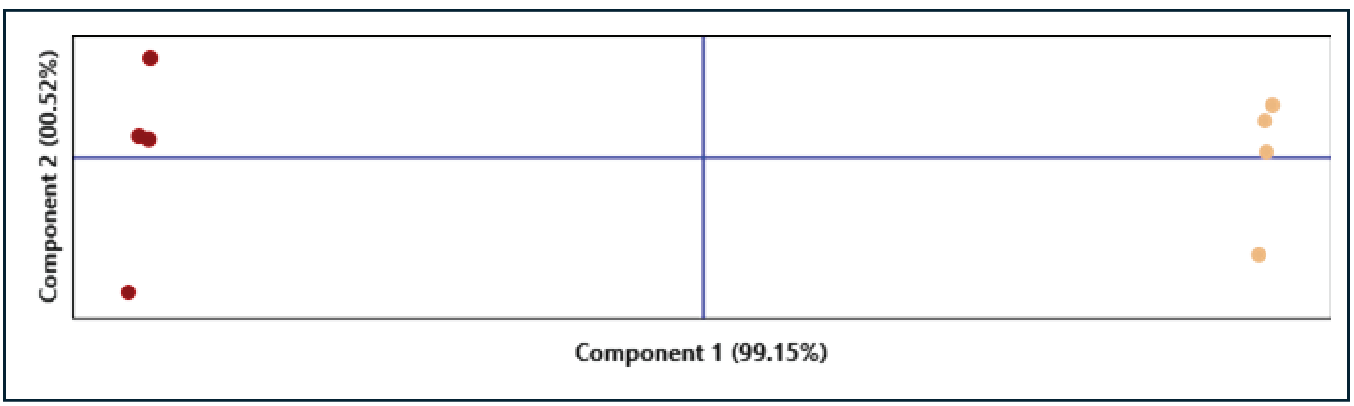

Figure 5. PCA Scores plot shows distinct clusters for the barrel-aged syrup (light brown) and the regular syrup (dark brown) with variables that differentiate the samples.

An examination of the loadings, shown in Figure 6, though, can be very helpful for uncovering the trends (higher in barrel-aged or higher in regular syrup) in the chemical changes. The bourbon barrel-aged syrups have positive scores on PC1 and the features that have positive loadings on PC1 are higher in the barrel-aged syrup, while the regular syrups have negative scores on PC1 and the features that have negative loadings on PC1 are observed at higher levels in the regular syrup. When the loadings are color coded by compound type, it is quite apparent that the trends (higher or lower in the barrel-aged sample) tended to be very consistent by functional group. Alcohols, esters, and alkanes were consistently observed at higher levels in the barrel-aged syrup while nitrogen-containing rings (pyrazines and pyridines), sulfur-containing compounds, aldehydes, and ketones were consistently observed at higher levels in the regular syrups. Oxygen-containing rings (furans and substituted furans) were higher or lower in the barrel-aged syrup on a per analyte basis.

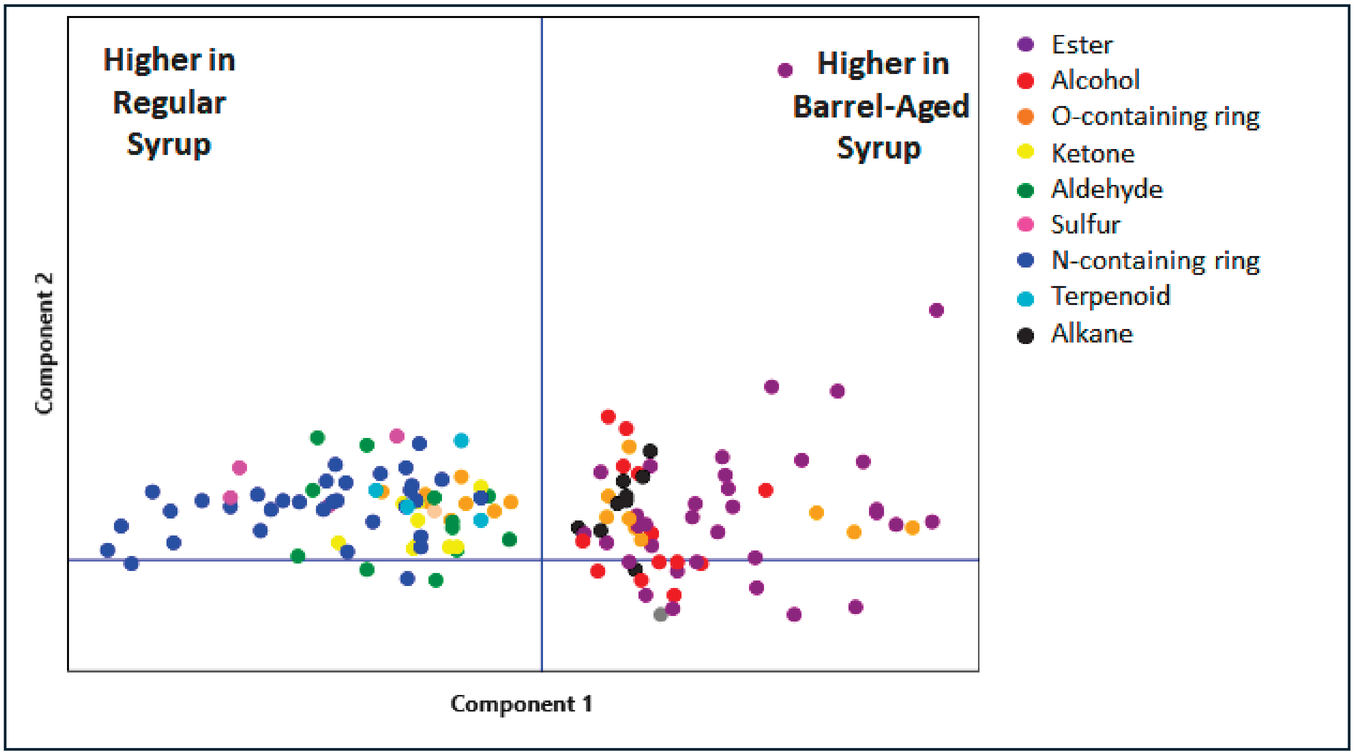

Figure 6. PCA loadings. Different compound classes tend to have different trends (increase or decrease) in the barrel-aged syrup compared to the regular syrup.

This characterization of the samples by compound class trends is helpful to understand the differences in the syrups. Information on specific individual analytes within these compound classes is also available and representative examples from various compound classes are shown in Figure 7.

Name	Formula	Similarity	CAS	Quant mass	R.I. calc	R.I. lib	R.I. Δ	Med RT1	Med RT2	1	2	3	4	5	6	7	8
Butanoic acid, ethyl ester	$C_6H_{12}O_2$	916	105-54-4	88	811	802	9	302.0	1.61	34	13	51	56	30	74	48	79
isobutyl acetate	$C_6H_{12}O_2$	913	110-19-0	73	783	772	11	274.0	1.59	37	33	50	34	57	10	30	31
isoamyl alcohol	$C_5H_{12}O$	917	123-51-3	55	742	736	6	236.0	1.79	36	34	10	15	74	15	39	17
isobutyl alcohol	$C_4H_{10}O$	924	78-83-1	74	639	624	15	160.0	1.59	16	31	36	56	76	36	39	38
1-Butanol, 2-methyl-	$C_5H_{12}O$	932	137-32-6	57	747	739	8	240.0	1.79	51	33	35	52	35	37	53	73
2-Acetyl-5-methylfuran	$C_7H_8O_2$	839	1193-79-9	109	1023	1039	-16	524.0	1.94	50	39	37	16	30	31	34	33
Furfural	$C_5H_4O_2$	919	98-01-1	96	843	833	10	338.0	0.10	31	74	73	79	31	53	31	33
2-Cyclopenten-1-one	C_5H_6O	845	930-30-3	82	845	832	13	340.0	0.16	57	51	57	36	31	34	36	15
Cyclopentanone	C_5H_8O	901	120-92-3	55	802	791	11	292.0	1.82	51	38	32	52	36	72	35	35
Butanal, 3-methyl-	$C_5H_{10}O$	917	590-86-3	44	666	652	14	176.0	1.58	35	34	33	38	34	15	16	37
Butanal, 2-methyl-	$C_5H_{10}O$	865	96-17-3	57	676	662	14	182.0	1.59	32	57	53	13	58	33	34	17
Disulfide, dimethyl	$C_2H_6S_2$	923	624-92-0	94	753	746	7	246.0	1.80	15	50	36	33	32	31	31	39
Dimethyl trisulfide	$C_2H_6S_3$	897	3658-80-8	126	981	971	10	482.0	1.95	38	32	17	58	32	38	52	34
Pyrazine	$C_4H_4N_2$	904	290-37-9	80	745	736	9	238.0	1.93	33	34	14	32	74	55	58	38
Pyrazine, methyl-	$C_5H_6N_2$	933	109-08-0	94	832	829	3	324.0	1.97	51	54	16	30	17	33	37	38

Figure 7. Peak identification metrics (similarity score, RI information) and relative trends (heat map color scale, barrel-aged syrup is columns 1-4 and regular syrup is columns 5-8) are shown for representative analytes from the various compound classes.

This workflow, that was facilitated by *ChromaTOF Tile*, rapidly uncovered the chemical differences between the two types of syrups, determined the identifications and trends for the specific chemical features associated with these differences, and uncovered specific functional group trends that may connect to the barrel-aging.

Conclusion

In this application note, GCxGC coupled to TOFMS was beneficial for separating the complex syrup samples and isolating the individual analytes. *ChromaTOF Tile* facilitated the sample class comparison and rapidly determined features in the data that differentiated the regular syrup and the barrel-aged syrup. The features were identified, and the trends were characterized, revealing consistent changes related to compound class. This collection of tools facilitates this type of non-targeted differential analysis and helps reveal more about your samples.

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