Application Note

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

Tracking the Chemical Profile of a Food Through the Spoilage Process with GCxGC and *ChromaTOF* Sync 2D

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Key Words: Tomato, Food Spoilage, Microbial Activity, Time Course, Process Monitoring, Solid Phase Microextraction, SPME, GC, GCxGC, MS, TOFMS, Retention Index, RI, Deconvolution, Peak Finding, Alignment, *ChromaTOF* Sync 2D, PCA

Introduction

There are many situations where it is important to understand the chemical information of a sample and how it changes through a process or over a time period. This information can provide good insight about a system and is useful for addressing a variety of questions and analysis objectives. For example, it may reveal the chemical reactions and the kinetics of the process. It may also indicate when a target state has been achieved, when a system is deviating from a target state, or how parameter variations impact the process. These types of detailed investigations can be important for optimization and understanding a wide range of systems.

In this work, we explore the process of food spoilage by tracking the degradation of tomatoes. While this is a specific look at a tomato, an understanding of general food deterioration is important as spoilage impacts the quality and safety of food products and has significant financial implications. Until a process like this is well understood, evaluating the chemical profile and the changes is generally a non-targeted discovery task. For food spoilage, many of the changes that occur will be reflected in the volatile and semi-volatile components of the sample. The aroma profile of the sample itself changes, and chemical markers of microbial activity are also likely to form.

GC and TOFMS are well-established techniques for these types of non-targeted characterizations of volatile and semi-volatile analytes and are well-suited for this type of investigation. Individual analytes are separated by GC, and TOFMS often provides the identification for these isolated analytes. The analyte coverage and sample characterization can be improved further by extending the analytical separation to two dimensions with comprehensive two-dimensional gas chromatography (GCxGC). GCxGC enhances the peak capacity and allows for more thoroughly exploring complex samples by determining more individual analytes. One of the challenges of interpreting this rich data, however, is linking analyte information across the multiple samples in order to determine the trends and patterns. Thus, software tools that compare sets of GCxGC samples are also useful for this type of analysis.

LECO's *ChromaTOF* Sync 2D offers advanced data processing options for handling these types of data sets. The software facilitates non-targeted characterization and comparisons by combining information for multiple GCxGC samples to a single peak table with full peak finding and deconvolution. This allows for effectively comparing analytes across the data set to find similarities, differences, and trends. The combination of GCxGC-TOFMS and *ChromaTOF* Sync 2D helped to reveal useful information about the tomato samples. In this case, the tools are demonstrated for monitoring the spoilage of a tomato sample, but they have broad applicability for many other process types.

Figure 1. The Pegasus BTX and ChromaTOF Sync 2D are used to compare a tomato sample through the spoilage process.

Experimental

A fresh garden tomato was pureed and left at room temperature for a week, allowing for natural spoilage. The tomato sample was analyzed right after pureeing and then daily through the time course. Triplicate samples were prepared each day by transferring 3 g of tomato to each 20 mL SPME vial. Each vial was sampled with HS-SPME by incubating for 2 min at 40 °C and extracting with the tri-phase fiber (PDMS, DVB, C-WR) for 5 min at the same temperature. The samples were then analyzed by GCxGC-TOFMS with LECO's *Pegasus* BTX 4D, as described in Table 1. An alkane standard was also analyzed with the same methods for retention index (RI) determinations.

Table 1. Instrument (*Pegasus* BTX 4D) Conditions

Auto Sampler	**LECO L-PAL 3 Autosampler**
Injection	Desorb for 2 min in GC inlet, split 10:1
Gas Chromatograph	**LECO QuadJet™ GCxGC**
Inlet	250 °C
Carrier Gas	He @ 1.4 mL/min
Column	Rxi-5ms, 30 m x 0.25 mm i.d. x 0.25 µm coating Rxi-17Sil MS, 0.6 m x 0.25 mm i.d. x 0.25 µm coating
Temperature Program	40 °C (hold 2 min), ramp 6 °C/min to 200 °C, ramp 24 °C/min to 250 °C Secondary Oven + 10 °C
Modulation	Quad jet thermal modulator, 2.5 s
Transfer Line	250 °C
Mass Spectrometer	**LECO *Pegasus* BT**
Ion Source Temperature	250 °C
Mass Range	35-500 m/z
Acquisition Rate	10 spectra/s (GC) and 100 spectra/s (GCxGC)

Results and Discussion

The time course samples, covering the spoilage process for a tomato, were analyzed with the *Pegasus* BTX 4D and *ChromaTOF* Sync 2D. This combination of GCxGC-TOFMS hardware and non-targeted peak finding and alignment software provided an efficient workflow for the exploration of the sample set. GCxGC-TOFMS generates rich chemical data for complex samples by combining enhanced two-dimensional chromatographic separations with full mass range MS detection. The BTX TOFMS, in particular, has excellent sensitivity, which can uncover additional low-level analytes. These capabilities lead to the separation and detection of more individual analytes in these complex samples. *ChromaTOF* Sync 2D provides nontargeted peak finding with deconvolution to efficiently process, align, and compile the chemical information for the entire sample set to get to the most useful information quickly. The approach uses GCxGC to address the sample complexity and *ChromaTOF* Sync 2D to address the data complexity. Representative GC and GCxGC chromatograms for one of the tomato samples are shown in Figure 2.

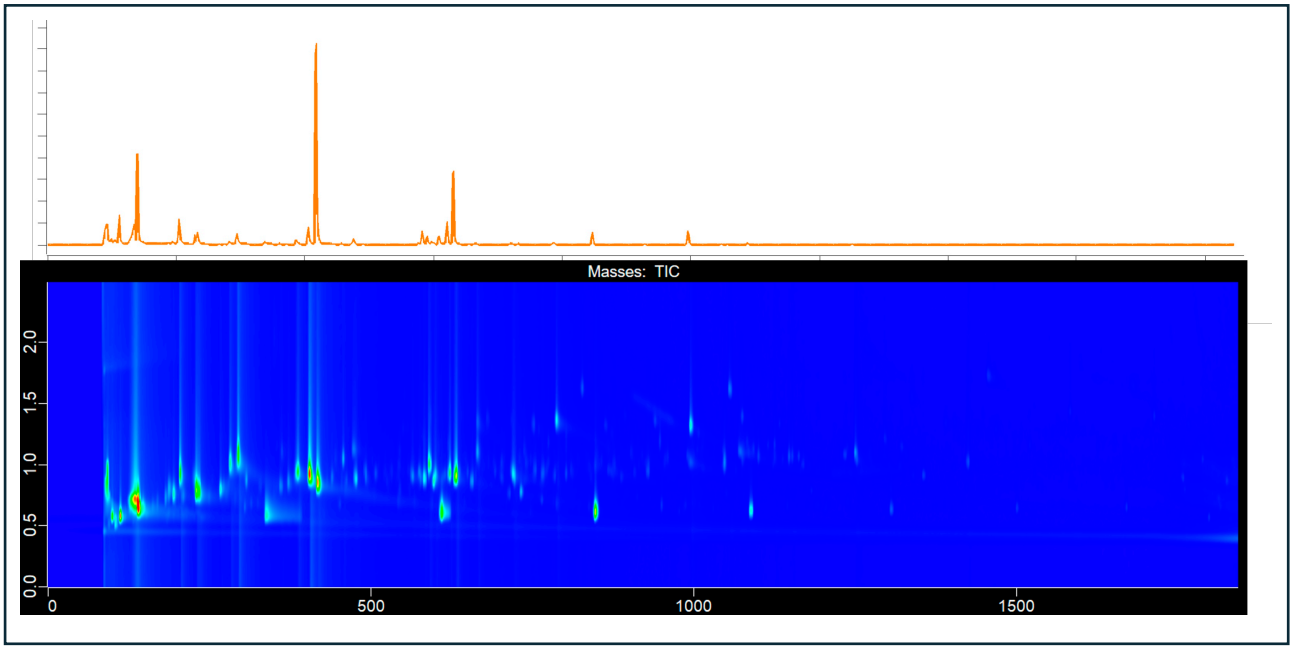

Figure 2. Representative GC and GCxGC chromatograms for tomato sample after 6 days.

The complexity of this sample is apparent. Using GCxGC-TOFMS was beneficial because the complementary second separation dimension increased the peak capacity and allowed for uncovering more chemical information. For example, there are many instances where peaks are vertically aligned in the contour plot in Figure 2. These are examples of coelution pairs that overlap with traditional GC and benefit from the additional separation dimension of GCxGC.

Some of the coelution pairs were separated by deconvolution in the GC data. For example, 3-methyl butanoic acid and 2-methylthio ethanol are shown in Figure 3. This coelution pair was deconvoluted in the GC data and chromatographically separated in the second dimension with GCxGC. The identifications were supported with spectral and RI matching to library databases, and both analytes were reliably determined with either GC or GCxGC.

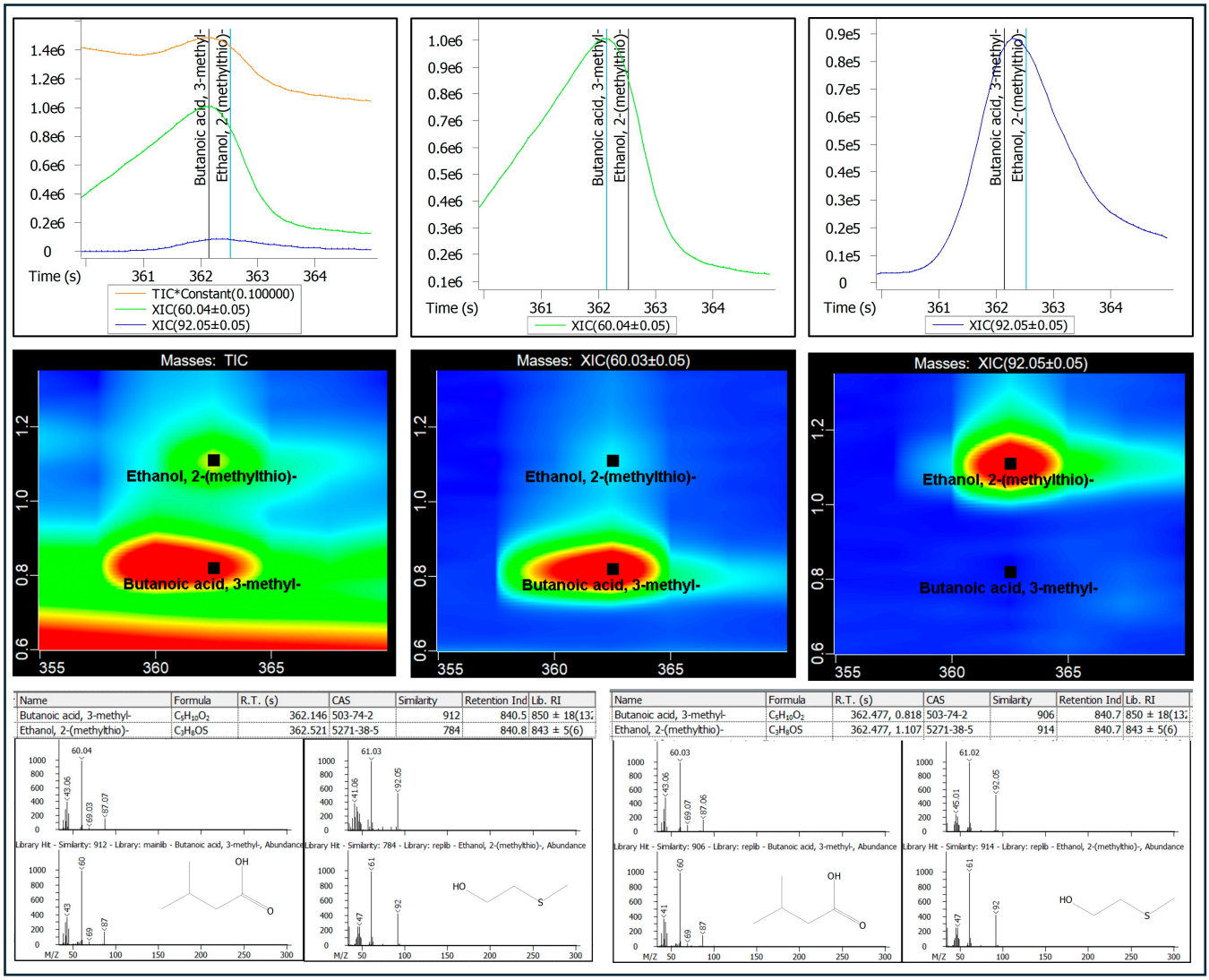

Figure 3. Some coelution pairs are deconvoluted with GC and chromatographically separated with GCxGC. Peak tables and spectral information are shown from the GC data (bottom left) and from the GCxGC data (bottom right).

Other coelution pairs, however, exceeded deconvolution capabilities in the GC data and were challenging to determine without GCxGC. For example, benzaldehyde and 1-ethyl-4-methyl benzene are shown in Figure 4. In this case, the two analytes were combined to a single peak marker and single spectrum in the GC data due to their complete chromatographic overlap. GCxGC separated the analytes in the second dimension leading to 2 separate peak markers. This improved the similarity score for benzaldehyde and uncovered information for 1-ethyl-4-methyl benzene, increasing the overall analyte coverage for these samples.

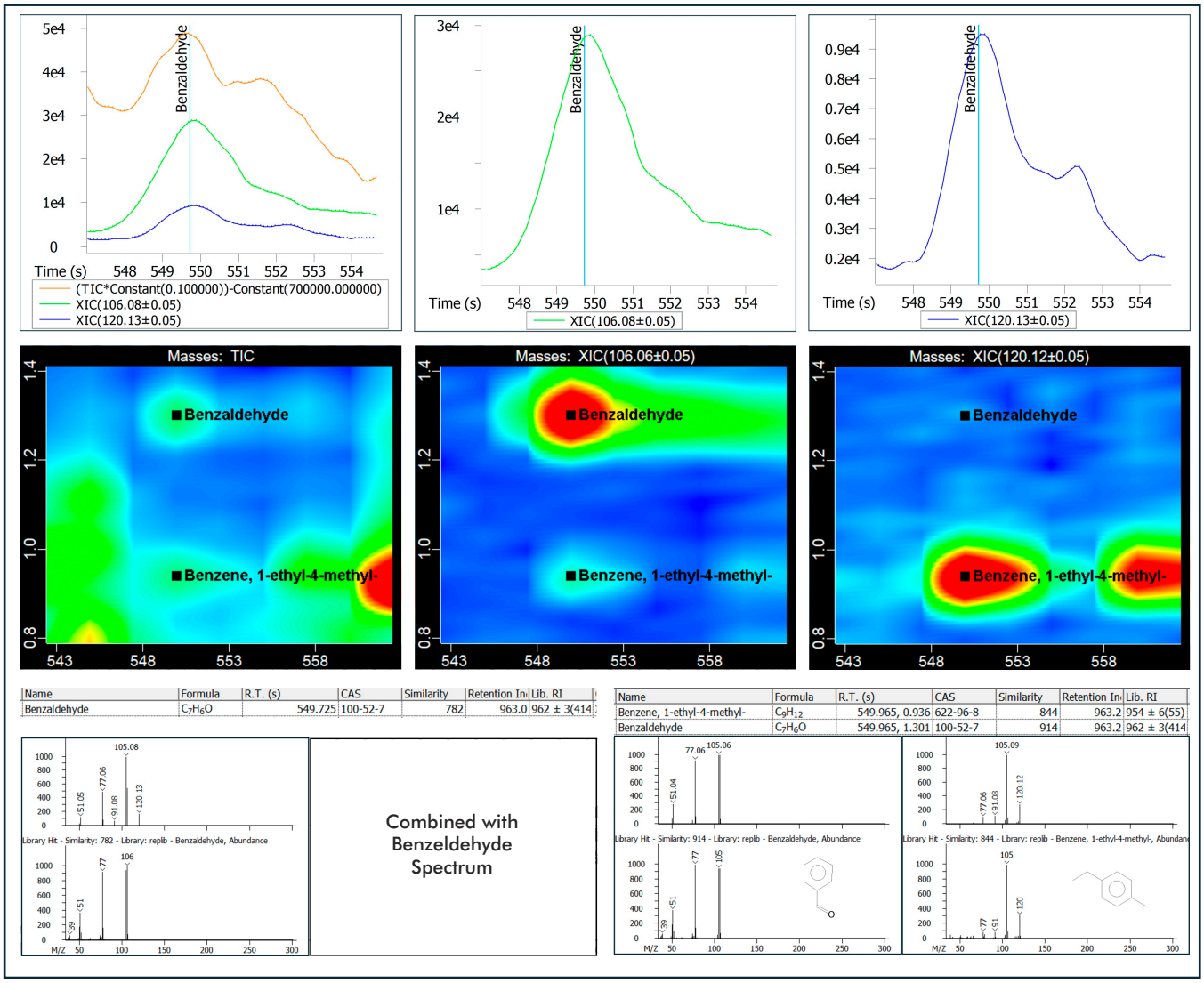

Figure 4. Some coelution pairs exceeded deconvolution with GC and needed the additional peak capacity of GCxGC to be determined. Peak tables and spectral information are shown from the GC data (bottom left) and from the GCxGC data (bottom right).

Given the non-targeted and discovery nature of this project, the improved chemical coverage with GCxGC was ideal. Analyzing the samples with GCxGC reduced the chance that an important or impactful analyte might be missed in the analysis.

Representative GCxGC contour plots for each of the time points are shown in Figure 5. From a visual comparison of these chromatograms, it is clear that the samples are complex and that many changes occurred during the spoilage process. Each time point would be a complex sample to analyze even if investigated independently, and analyzing all of the data together can be even more challenging. Having the individual analyte information compiled across the entire data set, though, is important in order to more thoroughly investigate the changes that are apparent and to look for trends in the data that are not visually apparent in the TIC. *ChromaTOF* Sync 2D streamlines these tasks by processing all samples together to generate a single combined peak table with tabulated peak areas for all samples.

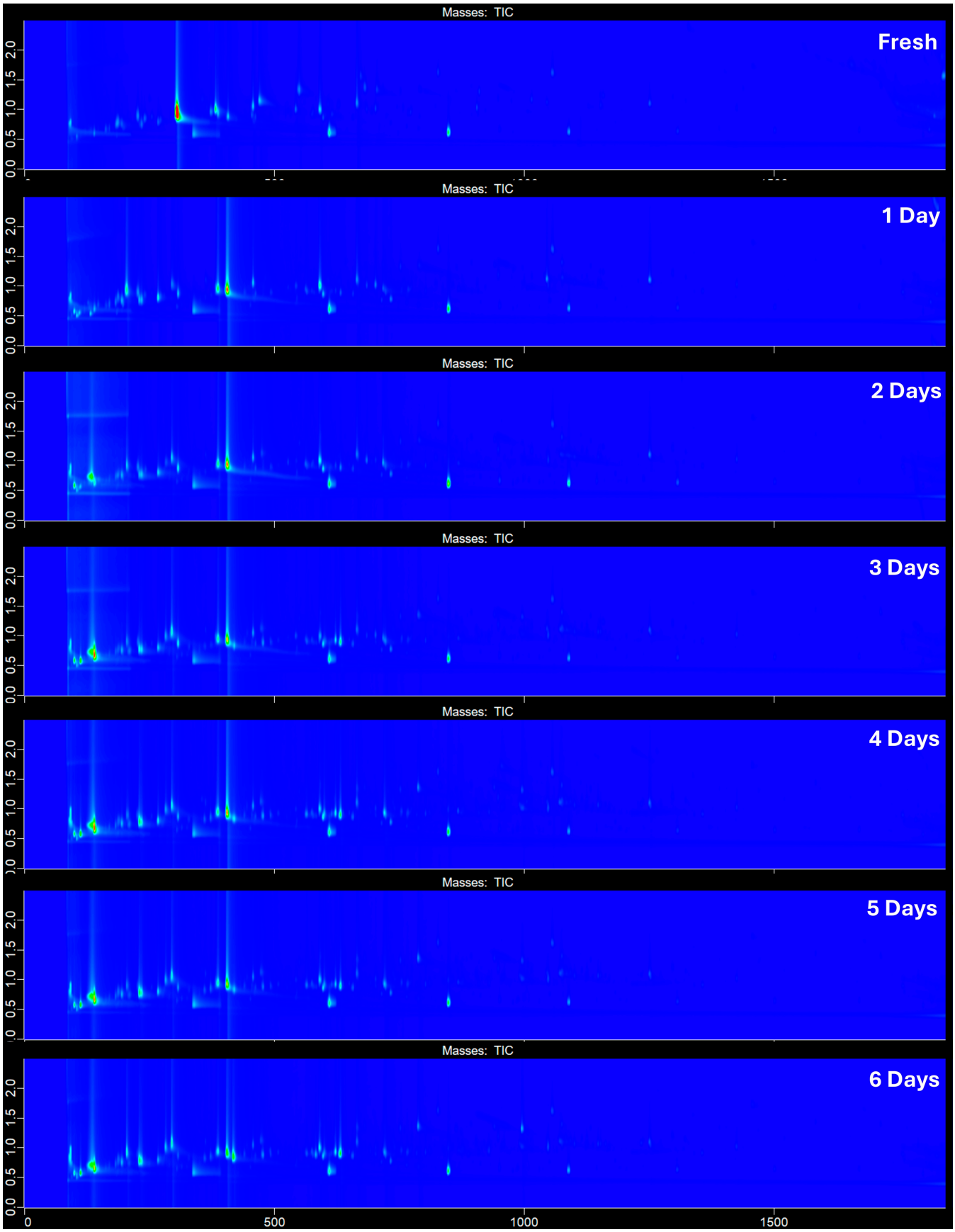

Figure 5. Representative contour plots for the tomato sample at each time point through the spoilage process.

For example, Table 2 shows a subset of the peak table with several representative analytes. The software incorporates deconvolution and can leverage both spectral data and RI information to determine analyte identifications. Identifications for all of the analytes in Table 2 are supported by spectral matching (similarity above 700) and have RI agreement with library databases, as indicated. Over 300 analytes met these identification criteria and were reliably determined. Peak areas from all samples were calculated with a consistent quant mass and compiled in the peak table, displayed as a heatmap in Table 2. The last 21 columns show the 7 time points in order with triplicate replicates of each. (Columns 1-3 are the fresh sample, columns 4-6 are after 1 day, columns 7-9 are after 2 days, columns 10-12 are after 3 days, columns 13-15 are after 4 days, columns 16-18 are after 5 days, and columns 19-21 are after 6 days.) The peak areas in the feature table can be shown as values or as a heatmap. This heatmap option provides a way to efficiently visualize the trends of many analytes together. Blue indicates low levels and red indicates higher levels, so color patterns that move from red to green/blue indicate analytes that decreased and color patterns that move from blue to green/red indicate analytes that increased over the time period. Aroma notes and descriptions for these features have also been added to the table to add more insight into these analytes and their trends.[1]

Table 2. Representative Analytes

Name	Formula	M.W.	Similarity	CAS	Quant ma	R.I. calc	R.I. lib	S/N	Aroma	Description	Med RI	Med RT2	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Hexanal	$C_6H_{12}O$	100	842	66-25-1	56.08	804	801	44394.69	green	fresh green fatty aldehydic grass leafy fruity sweaty	307.5	0.87																					
2-Hexenal	$C_6H_{10}O$	98	938	505-57-7	98.09	857	851	3678.56	green	sweet almond fruity green leafy apple plum vegetable	385.0	0.98																					
Methional	C_4H_8OS	104	810	3268-49-3	104.05	910	907	113.96	vegetable	musty potato tomato earthy vegetable creamy	465.0	1.28																					
Heptanal	$C_7H_{14}O$	114	935	111-71-7	96.12	904	901	808.98	green	fresh aldehydic fatty green herbal wine-lee ozone	455.0	0.91																					
Decanal	$C_{10}H_{20}O$	156	934	112-31-2	112.15	1209	1206	170.25	aldehydic	sweet aldehydic waxy orange peel citrus floral	922.4	0.99																					
Dimethyl trisulfide	$C_2H_6S_3$	126	927	3658-80-8	125.99	972	971	783.98	alliaceous	sulfurous cooked onion savory meaty	562.5	1.25																					
Butanoic acid	$C_4H_8O_2$	88	808	107-92-6	60.03	785	802	415.03	cheesy	sharp acetic cheese butter fruit	285.0	0.82																					
2,3-Butanedione	$C_4H_6O_2$	86	844	431-03-8	86.05	604	595	3413.94	buttery	strong butter sweet creamy pungent caramel	130.0	0.64																					
Octanoic acid, ethyl ester	$C_{10}H_{20}O_2$	172	727	106-32-1	88.07	1200	1196	417.30	waxy	fruity wine waxy sweet apricot banana brandy pear	909.9	0.94																					
Ethanol, 2-(methylthio)-	C_3H_8OS	92	780	5271-38-5	92.05	841	843	661.95	meaty	sulfurous meaty	362.5	1.10																					
2-Heptanone	$C_7H_{14}O$	114	876	110-43-0	114.13	894	891	381.24	cheesy	fruity spicy sweet herbal coconut woody	440.0	0.90																					
Ethanol	C_2H_6O	46	989	64-17-5	46.06	454	427	7259.30	alcoholic	strong alcoholic ethereal medicinal	100.0	0.58																					
1-Heptanol	$C_7H_{16}O$	116	889	111-70-6	70.09	973	970	2069.08	green	musty leafy violet herbal green sweet woody peony	565.0	0.91																					
1-Octanol	$C_8H_{18}O$	130	919	111-87-5	84.10	1072	1070	1653.21	waxy	waxy green orange aldehydic rose mushroom	720.0	0.92																					
Hexanoic acid, ethyl ester	$C_8H_{16}O_2$	144	733	123-66-0	88.07	1002	999	571.95	fruity	sweet fruity pineapple waxy green banana	610.0	0.88																					
Acetic acid	$C_2H_4O_2$	60	953	64-19-7	60.03	608	610	9025.01	acidic	sharp pungent sour vinegar	132.5	0.72																					
Pyrazine, 2-methyl-6-(1-propenyl)-,	$C_8H_{10}N_2$	134	748	18217-81-7	133.11	1103	1107	76.23			767.5	1.21																					
Pyrazine, 2,5-dimethyl-	$C_6H_8N_2$	108	902	123-32-0	108.09	917	917	1323.17	chocolate	cocoa roasted nuts roast beef woody grass medical	475.0	1.12																					
Pyrazine, 3-ethyl-2,5-dimethyl-	$C_8H_{12}N_2$	136	902	13360-65-1	136.13	1084	1081	259.61	nutty	potato cocoa roasted nutty	737.5	1.11																					
Acetic acid, methyl ester	$C_3H_6O_2$	74	818	79-20-9	74.05	537	526	14042.83	ethereal	ether sweet fruity	112.5	0.58																					
Acetic acid, pentyl ester	$C_7H_{14}O_2$	130	844	628-63-7	70.09	918	910	3468.17	fruity	ethereal fruity banana pear banana apple	477.5	0.88																					
Ethyl Acetate	$C_4H_8O_2$	88	852	141-78-6	88.06	620	612	9864.33	ethereal	ethereal fruity sweet weed green	140.0	0.66																					
Phenylethyl Alcohol	$C_8H_{10}O$	122	958	60-12-8	91.08	1117	1116	4259.33	floral	floral rose dried rose flower rose water	787.4	1.36																					
Propanoic acid, ethyl ester	$C_5H_{10}O_2$	102	909	105-37-3	102.09	716	710	1099.25	fruity	sweet fruity rum juicy fruit grape pineapple	207.5	0.74																					
Acetic acid, butyl ester	$C_6H_{12}O_2$	116	855	123-86-4	73.04	819	812	839.03	ethereal	ethereal solvent fruity banana	330.0	0.82																					
Linalool	$C_{10}H_{18}O$	154	831	78-70-6	93.09	1101	1099	821.14	floral	citrus floral sweet bois de rose woody green blueberry	765.0	0.93																					
1-Butanol, 2-methyl-	$C_5H_{12}O$	88	960	137-32-6	57.09	740	739	3219.00	ethereal	ethereal fusel alcoholic fatty greasy winey whiskey leathery	235.0	0.77																					
1-Butanol, 3-methyl-	$C_5H_{12}O$	88	921	123-51-3	55.07	736	736	15667.28	fermented	fusel oil alcoholic whiskey fruity banana	230.0	0.78																					
1-Hexanol, 4-methyl-	$C_7H_{16}O$	116	755	818-49-5	70.09	948	953	270.04	sweaty	sweaty	525.0	0.87																					
Benzeneacetic acid, methyl ester	$C_9H_{10}O_2$	150	760	101-41-7	150.11	1183	1178	60.50	honey	sweet floral honey spice waxy almond	884.9	1.37																					
Methyl valerate	$C_6H_{12}O_2$	116	723	624-24-8	74.05	828	823	300.89	fruity	sweet green fruity apple pineapple nutty	342.5	0.83																					
Butanoic acid, 3-methyl-	$C_5H_{10}O_2$	102	713	503-74-2	60.03	841	850	1462.90	cheesy	sour stinky feet sweaty cheese tropical	362.5	0.81																					
Hexanoic acid	$C_6H_{12}O_2$	116	938	142-62-1	73.04	980	990	939.25	fatty	sour fatty sweat cheese	575.0	0.93																					
Acetic acid, 2-phenylethyl ester	$C_{10}H_{12}O_2$	164	936	103-45-7	104.09	1261	1258	7261.78	floral	floral rose sweet honey fruity tropical	994.9	1.32																					
Acetic acid, heptyl ester	$C_9H_{18}O_2$	158	887	112-06-1	98.13	1115	1112	854.61	green	fresh green rum ripe fruit pear apricot woody	784.9	0.91																					
Acetic acid, hexyl ester	$C_8H_{16}O_2$	144	766	142-92-7	101.08	981	1011	552.09	fruity	fruity green apple banana sweet	577.5	0.88																					
Acetic acid, phenylmethyl ester	$C_9H_{10}O_2$	150	897	140-11-4	108.08	1169	1164	524.08	floral	sweet floral fruity jasmin fresh	864.9	1.31																					
Acetic acid, octyl ester	$C_{10}H_{20}O_2$	172	821	112-14-1	112.15	1214	1210	445.18	floral	green earthy mushroom herbal waxy	929.9	0.94																					

Individual analytes can be explored further from the feature table with additional visualization plots and options. For example, detailed information for hexanal, the first line in Table 2, is shown in Figure 6. Hexanal was identified with a spectral similarity score of 842 and the deconvoluted spectrum and library match are shown in the top left corner. The observed or calculated RI value of 804 also has good agreement with the library database value of 801, shown in the table. The red to blue trend in the heat map and the bar chart of peak areas clearly indicate that this analyte was at a high level in the fresh sample and rapidly decreased after the first day. The aroma notes for hexanal are fresh and green, so this decrease is likely important in understanding how the aroma characteristics of the tomato change. This trend was also visible in the chromatograms shown in Figure 5. Hexanal is the major analyte that elutes around 300 s in the top contour plot. It is apparent in the fresh sample, but not at the later time points. *ChromaTOF* Sync 2D efficiently compiled and tabulated the data to describe this observation.

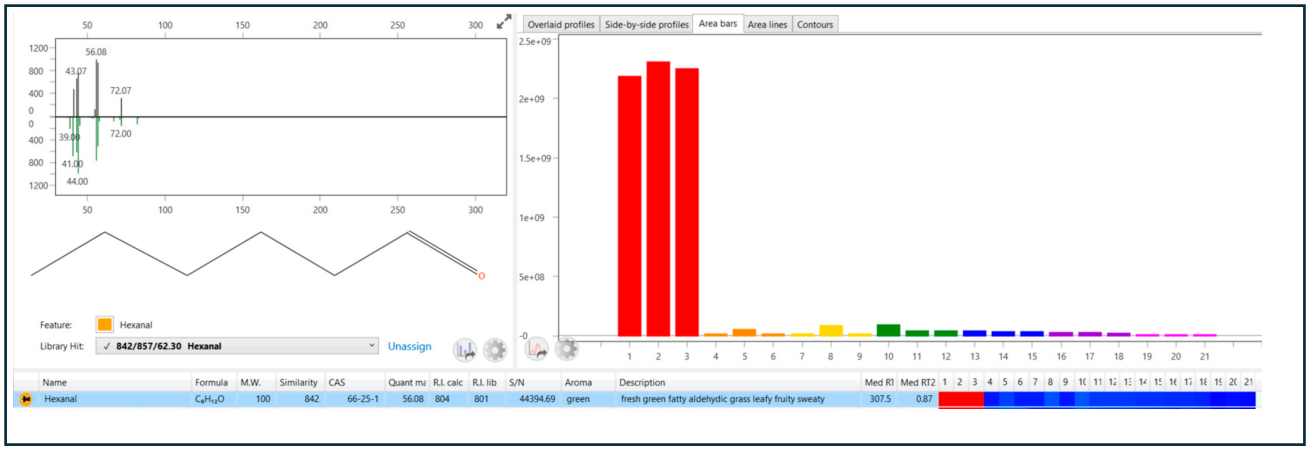

Figure 6. Hexanal was determined with Pegasus BTX 4D and ChromaTOF Sync 2D. The analyte is observed at a high level in the fresh sample and then quickly diminishes.

Other specific analytes can also be reviewed from this compiled data to gain more insight into the spoilage process and the aroma changes. For example, the analytes in the coelution pair shown in Figure 3 are also shown in Table 2. Both of these analytes increased over time, and more detailed information is shown in Figures 7 and 8. 2-(methyl thio)-ethanol, shown in Figure 7, has sulfurous and meaty aroma descriptors. This analyte increased after the first day and then remained at a consistent level. 3-methyl butanoic acid, shown in Figure 8, has cheesy, sour, stinky, and sweaty aroma notes. This analyte increased steadily over the week. These types of analytes likely contributed to the increasingly unpleasant aroma of the sample.

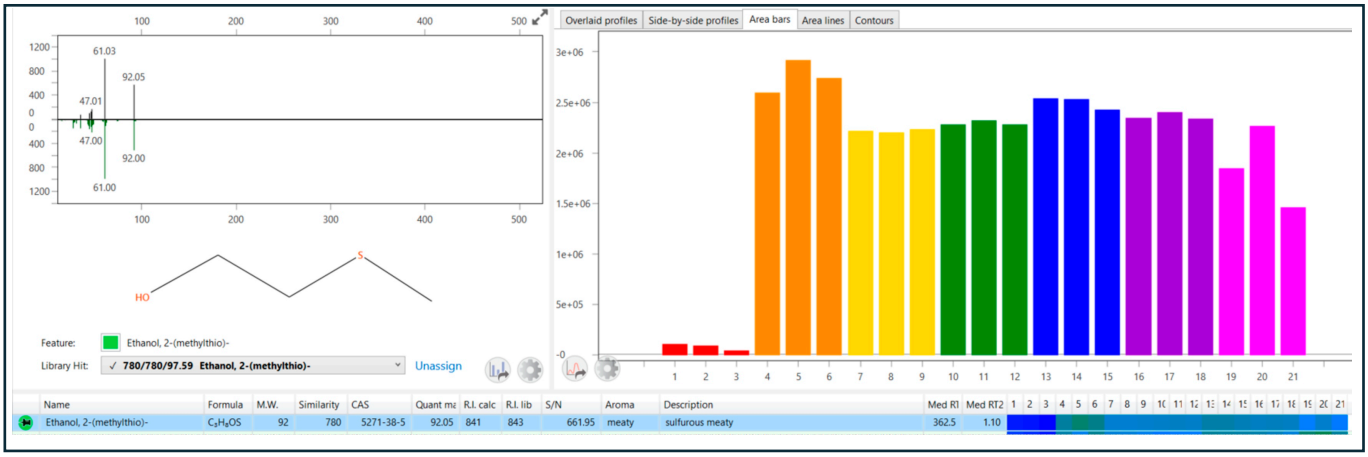

Figure 7. 2-(methylthio)-ethanol was determined in the food spoilage samples with Pegasus BTX 4D and ChromaTOF Sync 2D.

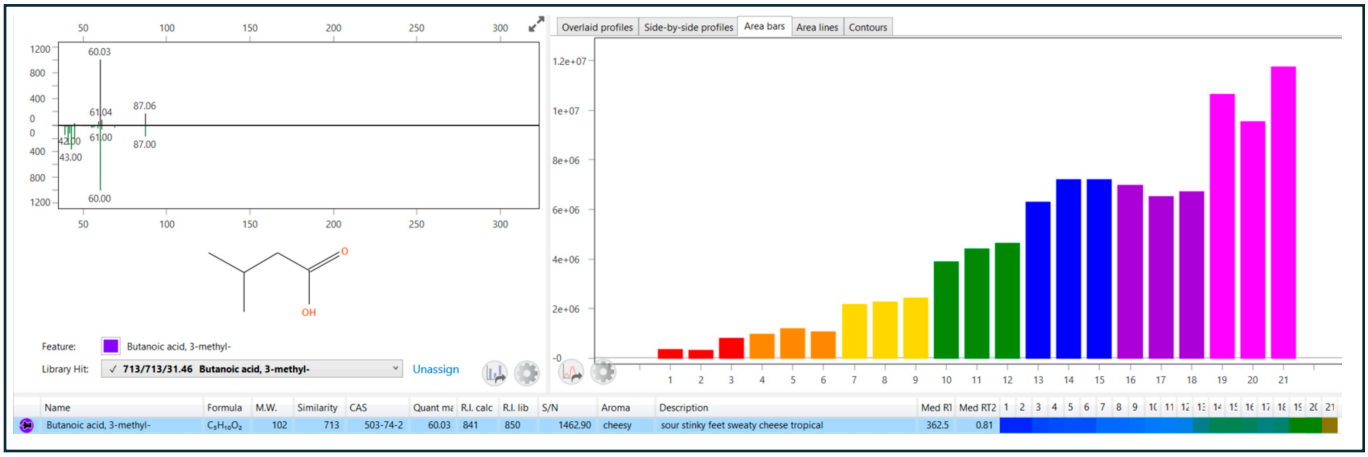

Figure 8. 3-methyl butanoic acid was determined in the food spoilage samples with Pegasus BTX 4D and ChromaTOF Sync 2D.

In addition to changing aromas, there are also likely indicators of microbial activity. For example, ethanol, shown in Figure 9, was measured after the first day, and acetic acid, shown in Figure 10, was observed the following day. Identifying these analytes and determining the timing of their trends can improve the understanding of the overall process.

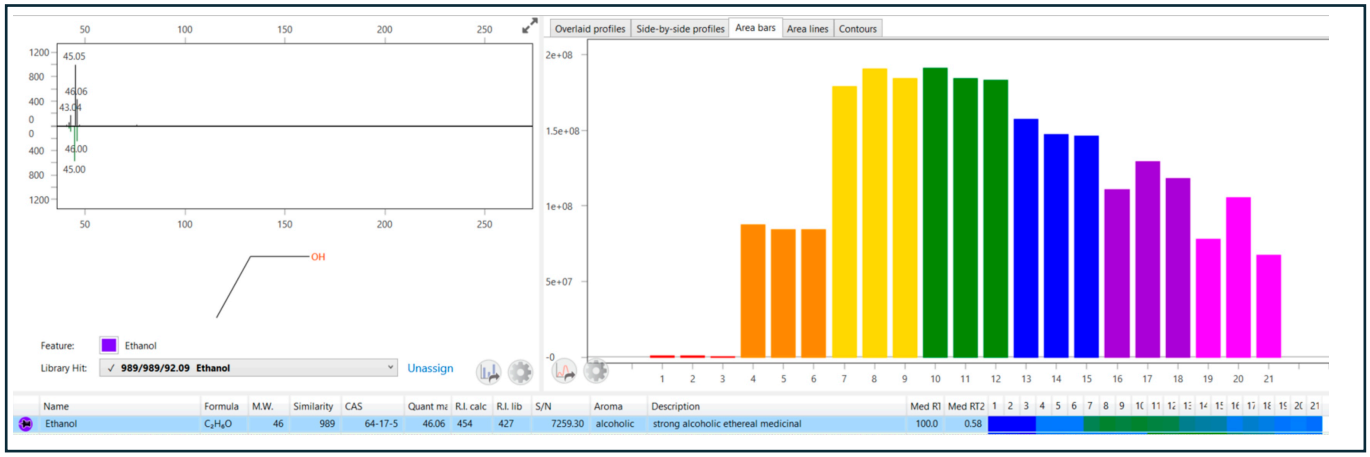

Figure 9. Ethanol was determined in the food spoilage samples with Pegasus BTX 4D and ChromaTOF Sync 2D.

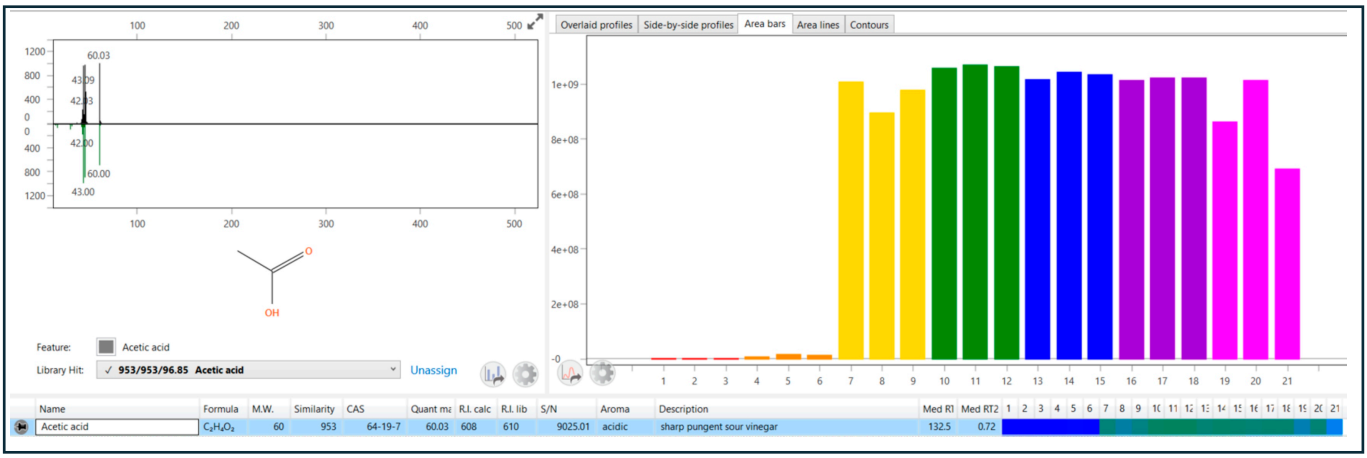

Figure 10. Acetic acid was determined in the food spoilage samples with Pegasus BTX 4D and ChromaTOF Sync 2D.

From the contour plots in Figure 5, the heatmap summary view of Table 2, and the examples in Figures 6-10, it is clear that this is a complex system with many chemical changes throughout this process. Some analytes decreased while others increased. The trends and timing of these general directions of change were also largely variable, as evident in these examples. Some analytes increased early with high levels after the first day and others did not start to increase until several days into the process. Some analytes increased abruptly, while others had a gradual and steady increase. The trend after increasing was also variable. Some maintained an elevated level, while others started to decrease again. Some of these overall trends in the data can be explored and summarized with additional comparison options in *ChromaTOF* Sync 2D.

For example, principal component analysis (PCA), is shown in Figure 11. In the scores plot, each sample is represented as a data point and the proximity of the sample scores to each other indicates overall trends. Those that are closest to each other are more similar and those that are further apart are more distinct. It can be noted that the fresh samples are separated more on PC1 from the later time points. This indicates that the fresh sample is more chemically distinct, suggesting that there were many chemical changes that occurred early in this process on that first day. It can also be noted that the other samples move along PC1 in time order. The samples collected after 1, 2, and 3 days each group independently and the last 3 days all cluster together. This suggests the presence of time trends and also that the samples started to have more chemical similarities to each other at the later stages of the process. If this were a process with a target end point, this type of analysis could provide insight as to when the changes were slowing or when that target point had been achieved.

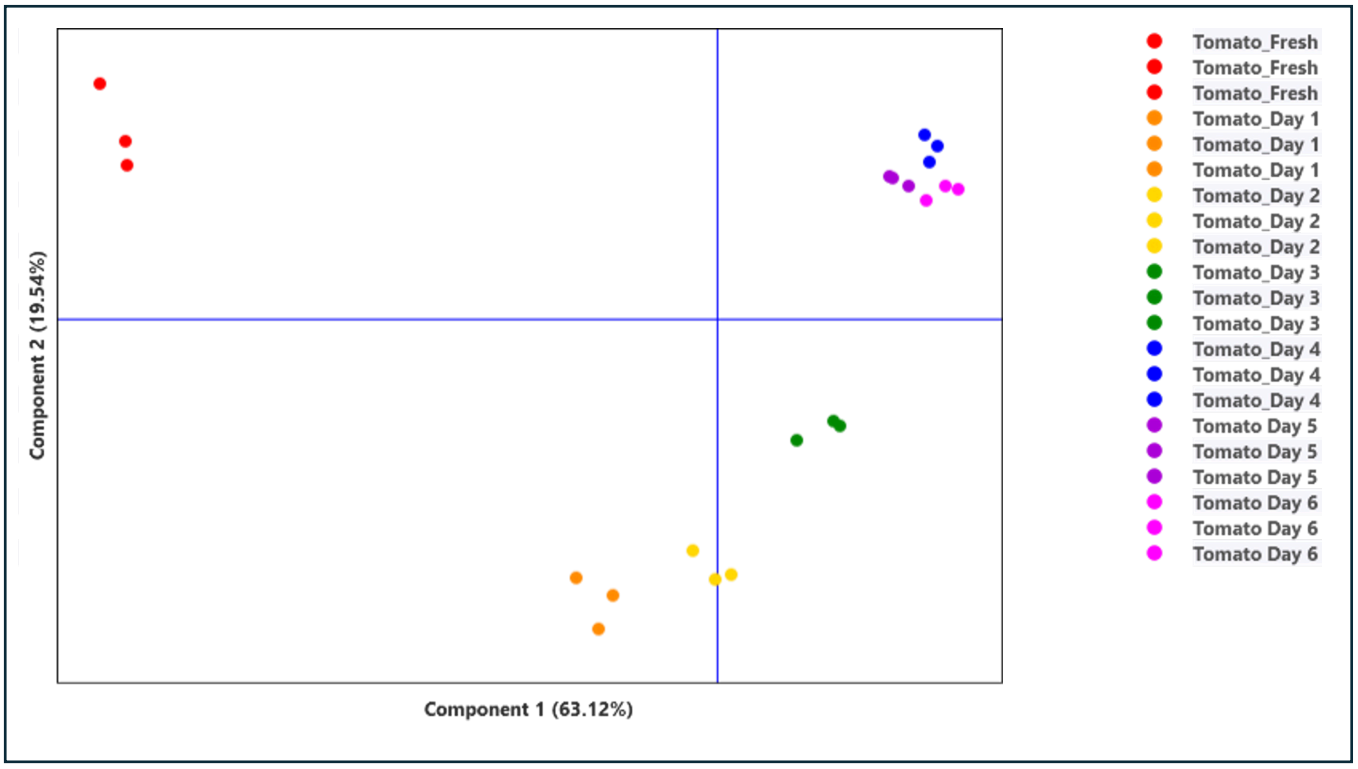

Figure 11. PCA Scores of all the tomato samples through the spoilage process.

Conclusion

In this work, LECO's GCxGC-TOFMS *Pegasus* BTX 4D and *ChromaTOF* Sync 2D were used to explore the spoilage process of a tomato sample. GCxGC was crucial for separating the analytes within this complex sample and the *Pegasus* BTX TOFMS provided powerful detection capabilities, with full m/z range data and excellent sensitivity. *ChromaTOF* Sync 2D streamlined the data interpretation with full non-targeted peak finding and alignment of the sample set data, which connected and compiled peak information for the full data set. Many analyte trends and differences were observed across the time course, and the analytical tools used in this work were essential for efficiently uncovering them. Additional analysis options in *ChromaTOF* Sync 2D, like PCA, help provide more insight to the overall trends in the data. These tools facilitate and simplify this type of non-targeted characterization. While this is a focused look at tomato spoilage, the workflow demonstrated here is also broadly applicable for investigating other types of processes and other types of non-targeted work.

References

[1]Good Scents database, www.thegoodscentscompany.com

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