# Application Note

## *Instrument: Pegasus® BT*

# Chemical Aroma Profile of Dark Chocolate Bar

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Key Words: Aroma profile, HS-SPME, GC-MS, Time-of-Flight, Chocolate

## *Introduction*

Understanding the aroma characteristics and the specific chemicals associated with these aromas in a complex sample of interest can be beneficial for a wide range of purposes in the food, flavor, and fragrance market. Among other objectives, detailed chemical information can be used for quality control, process optimization, reverse engineering of desired aroma characteristics, and better understanding of a product or sample of interest. Gas chromatography with mass spectrometry (GC-MS) is a good choice for determining specific chemical information because individual analytes within a complex mixture are routinely separated, identified, and quantified. These individual analytes can be linked with their specific aroma properties to reveal this complete chemical aroma characterization for samples of interest. Coupling headspace solid phase micro-extraction (HS-SPME) sampling with GC-MS provides a complete workflow from sample preparation through data analysis for these types of studies. HS-SPME is a sampling technique that collects the volatile and semi-volatile analytes in the headspace of a sample of interest. Gas chromatography separates these analytes from each other and background, and MS detects the analytes for identification and relative quantification information. Here, we describe how the information provided by this analytical technique and the automated processing tools can lead to complete chemical aroma characterization of a chocolate sample.

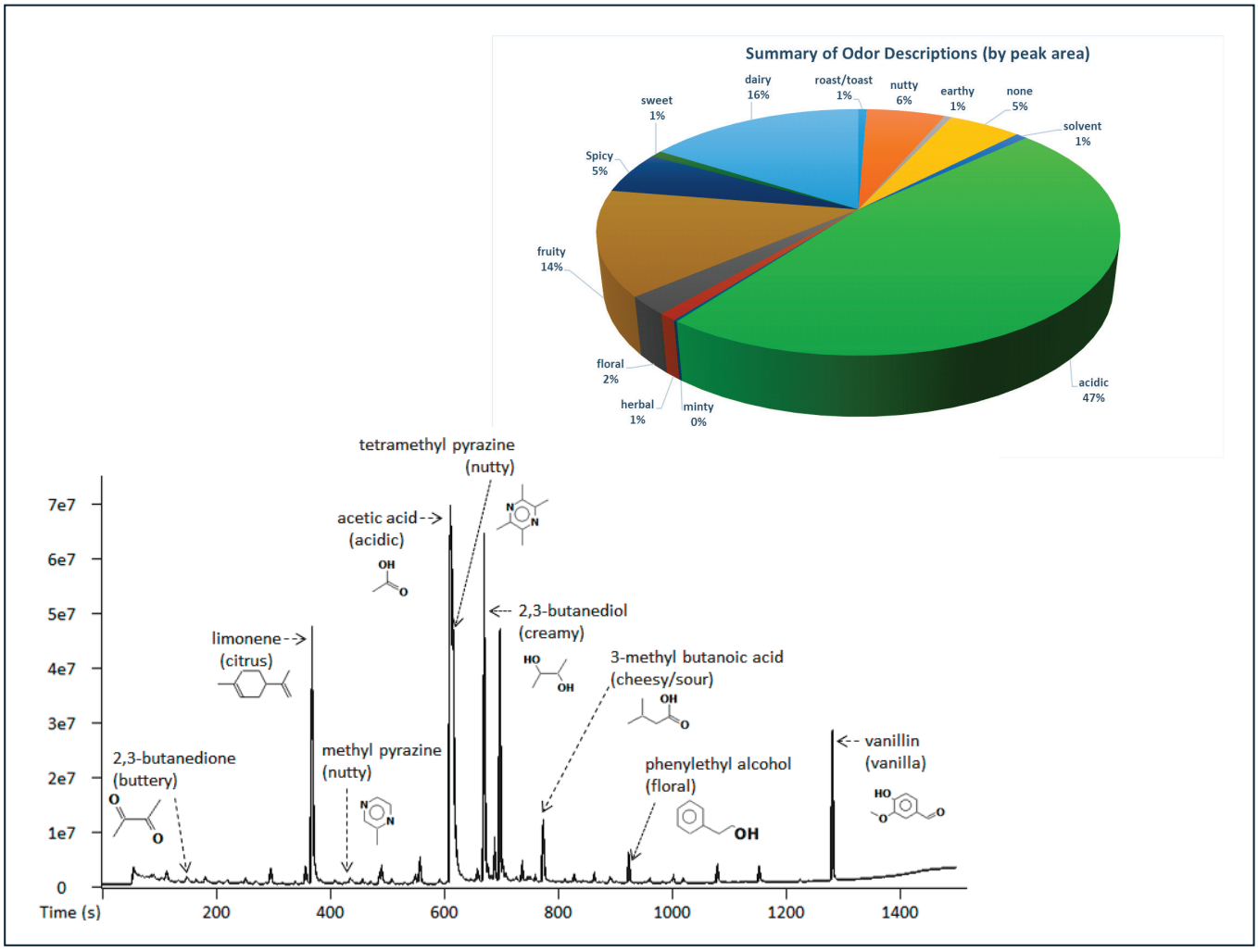



***Figure 1. Chocolate Total Ion Chromatogram (TIC) with representative analytes highlighted. The associated chemical aroma summary for the sample is also shown in the form of a pie chart.***

## Experimental

A chocolate sample was analyzed by HS-SPME with GC-MS using method parameters described in Table 1. The samples were incubated for 5 min at 60 °C and extracted for 15 min at 60 °C with a DVB/CAR/PDMS fiber (Supelco). Immediately after extraction, the SPME fiber was exposed in the GC-inlet for 3 minutes at a temperature of 250 °C for desorption and injection. The fiber was conditioned for 5 minutes at 250 °C between each sample. The HS-SPME procedure was fully automated with LECO's L-PAL3 Autosampler. Data for an alkane standard was also collected with the same methods, allowing for Retention Index determinations.

**Table 1. GC-TOFMS (*Pegasus* BT) Conditions**

| **Gas Chromatograph** | **Agilent 7890 with LECO L-PAL 3 Autosampler** |
|---|---|
| Injection | SPME, 3 min desorption in 250 °C inlet, split 10:1 |
| Carrier Gas | He @ 1.4 mL/min |
| Column | Stabilwax, 30 m x 0.25 mm i.d. x 0.25 µm coating (Restek) |
| Temperature Program | 3 min at 40 °C, ramped 10 °C/min to 250 °C, hold 1 min |
| Transfer Line | 250 °C |
| **Mass Spectrometer** | **LECO *Pegasus* BT** |
| Ion Source Temperature | 250 °C |
| Mass Range | 33-600 m/z |
| Acquisition Rate | 10 spectra/s |

## Results and Discussion

A representative chromatogram and summary of the chemical aroma profile for a chocolate sample are shown in Figure 1. GC-MS is a powerful tool for this type of characterization of a sample because it readily provides detailed information on the chemical makeup of a product. The pie chart summarizes the overall aroma properties for this chocolate sample with major odor descriptors of acidic, dairy, fruity, floral, spicy, nutty, and roasted or toasted. These descriptors and their contribution to the chemical profile overall come from the determination of chemical information at the individual analyte level, with some representative analytes also indicated. This analytical approach offers two mechanisms of separation to help isolate more individual analytes present in the sample and to better characterize the profile. The primary separation occurs with chromatography. Mathematical deconvolution of the full mass-range TOFMS data then further separates analytes that may still coelute even after the chromatographic separation. The mathematical deconvolution provides clean spectral data per analyte that can be searched against library databases for tentative identifications. Retention index determinations add confidence to these identifications and once identified the associated aroma properties can be compiled to understand the overall aroma profile that the chemical data provides. Sensory descriptors were obtained from The Good Scents Company website.[1]

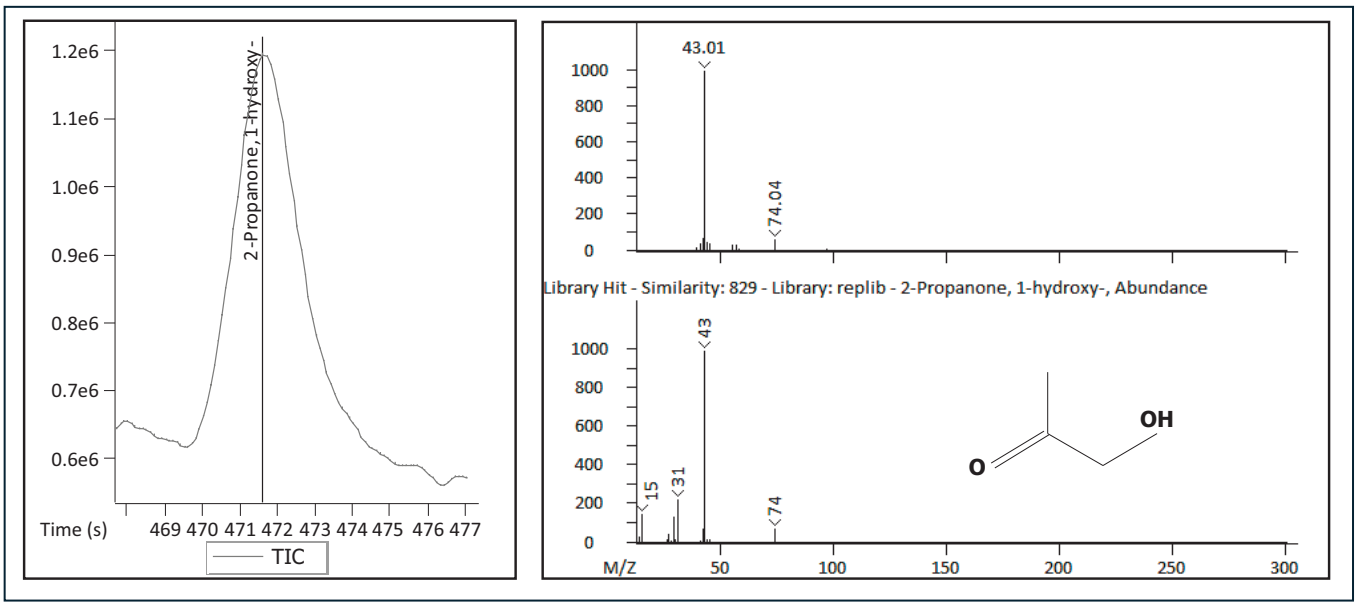



***Figure 2. Information for a chromatographically isolated analyte.***

Examples of the determinations of individual analytes are shown in Figures 2 and 3. In Figure 2, 1-hydroxy 2-propanone separates chromatographically from other analytes in this complex mixture. The mass spectral data acquired with this instrument (top) matched to NIST (bottom) with a similarity of 829 out of 1000. The retention index information further supported this identification with observed RI = 1298.7 compared to library RI =1303. Once confidently identified, the aroma characteristics for this analyte were determined. In this case, the identified analyte contributed caramel odor characteristics to the sample. When analytes chromatographically coelute, deconvolution adds separation and can often provide clean spectra, as shown in Figure 3. Here, 1-(2-furanyl) ethanone and 3,5-diethyl-2-methyl pyrazine are mathematically separated and determined from matching the deconvoluted spectra and retention index information to library databases. The analytes match to the library information with similarity scores of 888 and 853 for 1-(2-furanyl) ethanone and 3,5-diethyl-2-methyl pyrazine, respectively. Retention index supported both identifications with calculated values of 1506.3 and 1507.7 compared to library values of 1499 and 1496, respectively. Once confidently identified, the aroma characteristics of balsamic and nutty were determined.

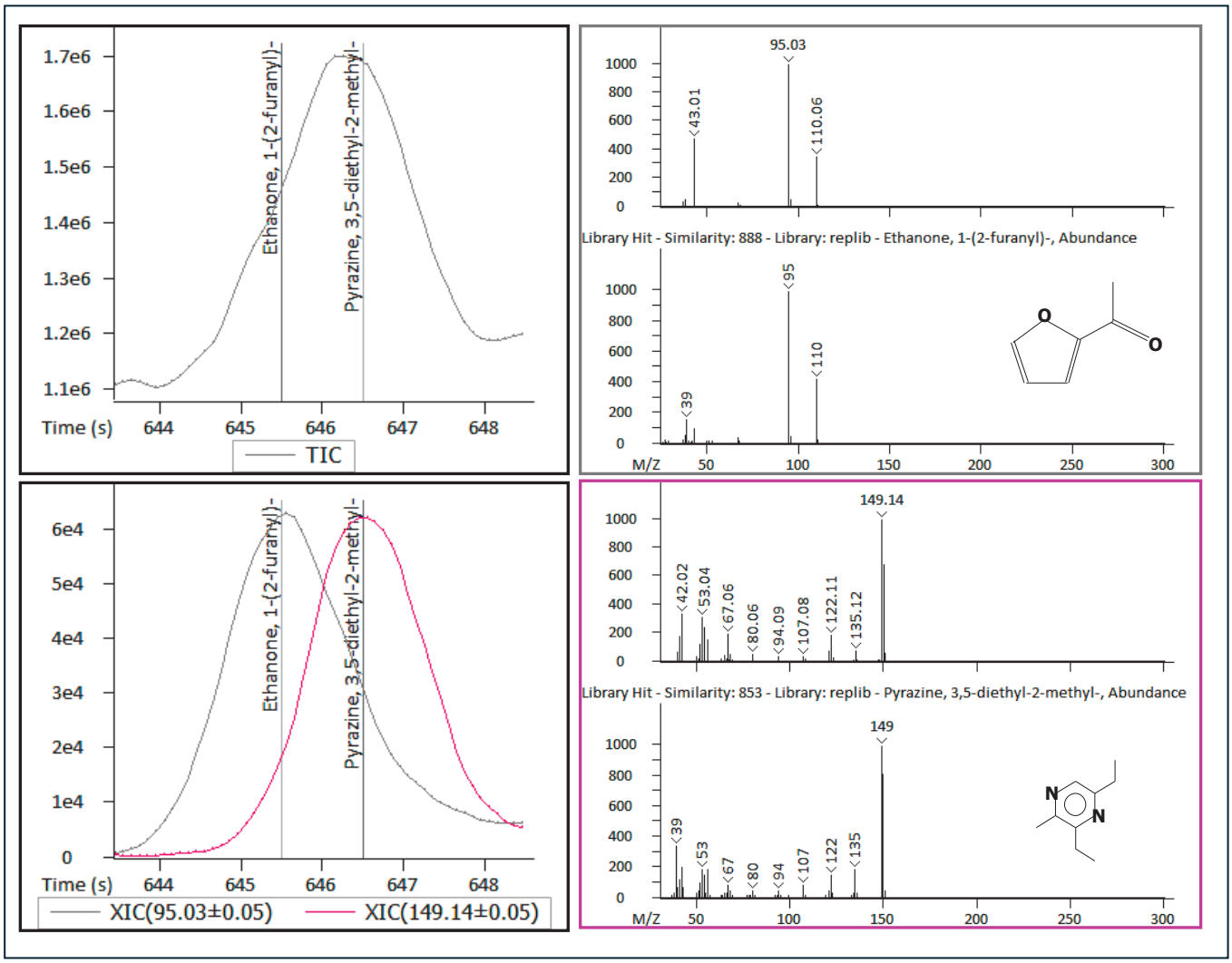



***Figure 3. Deconvolution provides information on analytes that chromatographically coelute.***

Once analytes in the sample are isolated, identified through these processing tools, and linked to the associated aroma characteristics, the chemical aroma profile of the sample can be characterized overall, as shown in Figure 1. To connect peak area to a sensory detection directly, both the response factor for that analyte on the instrument and the sensory threshold would be required. In the absence of these values, the peak area can still generate a chemical profile for aroma characterization. LECO's deconvoluted TIC provides a reasonably consistent measure of peak area that can be useful for this comparison. The deconvoluted TIC is the sum of all spectral peaks in the deconvoluted peak true spectrum, integrated over the concentration profile for the chromatographic peak. For an intense chromatographically isolated analyte, the deconvoluted TIC is similar to the TIC, only removing some baseline and background noise. Using the deconvoluted TIC is preferable to using the TIC, however, because it accounts for coelution and can better determine low-level analytes that may be buried in background noise in the TIC. Using the deconvoluted TIC is also preferable to using an individual mass per analyte because of the better consistency between analytes. Examples of the TIC compared to the deconvoluted TIC for these scenarios are shown in Figure 4.

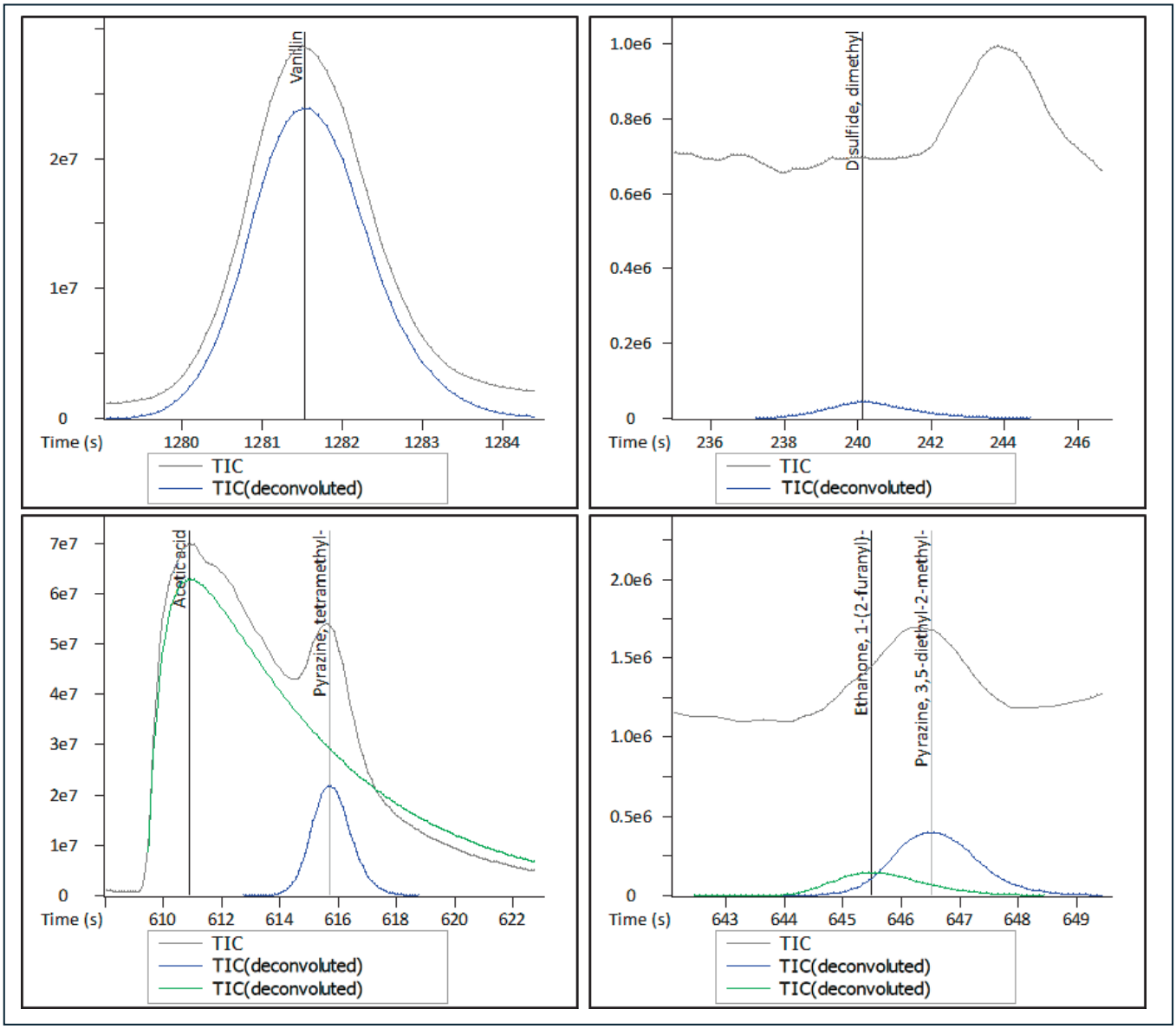
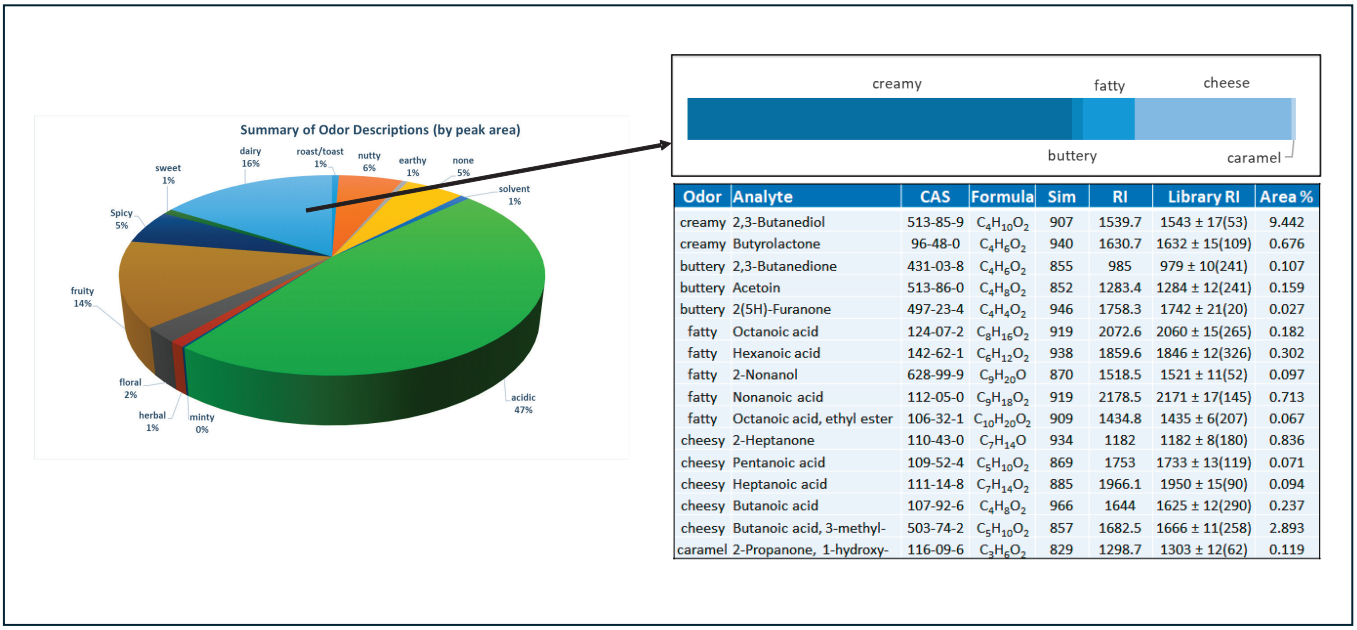


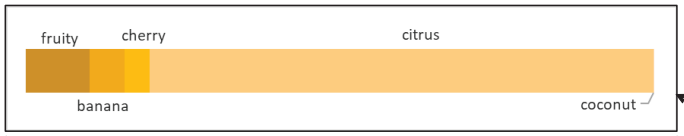

***Figure 4. Deconvoluted TIC, from the ChromaTOF® brand software, removes background noise, baseline, and interferences from coeluting analytes to provide more reliable peak areas.***

The area for each analyte in the sample was automatically calculated with the deconvoluted TIC in *ChromaTOF*. The complete chemical aroma profile pie chart was then determined by grouping the analytes by odor type and determining the total peak area for that odor type by summing the peak areas of all the individual analytes. Analytes with S/N > 100 that were identified with similarity scores > 700 and confirmed by retention index were included in the tabulated results. The pie chart in Figure 1 shows this summary. For a given odor type, more specific odor descriptors were also available and detailed aroma properties were further explored. For example, an expansion of the analytes with dairy odors is shown in Figure 5 and an expansion of the analytes with fruity odors is shown in Figure 6. The breakdown of various types of fruity aromas can be observed, for example.




| Odor | Analyte | CAS | Formula | Sim | RI | Library RI | Area % |
|---|---|---|---|---|---|---|---|
| creamy | 2,3-Butanediol | 513-85-9 | $C_4H_{10}O_2$ | 907 | 1539.7 | 1543 ± 17(53) | 9.442 |
| creamy | Butyrolactone | 96-48-0 | $C_4H_6O_2$ | 940 | 1630.7 | 1632 ± 15(109) | 0.676 |
| buttery | 2,3-Butanedione | 431-03-8 | $C_4H_6O_2$ | 855 | 985 | 979 ± 10(241) | 0.107 |
| buttery | Acetoin | 513-86-0 | $C_4H_8O_2$ | 852 | 1283.4 | 1284 ± 12(241) | 0.159 |
| buttery | 2(5H)-Furanone | 497-23-4 | $C_4H_4O_2$ | 946 | 1758.3 | 1742 ± 21(20) | 0.027 |
| fatty | Octanoic acid | 124-07-2 | $C_8H_{16}O_2$ | 919 | 2072.6 | 2060 ± 15(265) | 0.182 |
| fatty | Hexanoic acid | 142-62-1 | $C_6H_{12}O_2$ | 938 | 1859.6 | 1846 ± 12(326) | 0.302 |
| fatty | 2-Nonanol | 628-99-9 | $C_9H_{20}O$ | 870 | 1518.5 | 1521 ± 11(52) | 0.097 |
| fatty | Nonanoic acid | 112-05-0 | $C_9H_{18}O_2$ | 919 | 2178.5 | 2171 ± 17(145) | 0.713 |
| fatty | Octanoic acid, ethyl ester | 106-32-1 | $C_{10}H_{20}O_2$ | 909 | 1434.8 | 1435 ± 6(207) | 0.067 |
| cheesy | 2-Heptanone | 110-43-0 | $C_7H_{14}O$ | 934 | 1182 | 1182 ± 8(180) | 0.836 |
| cheesy | Pentanoic acid | 109-52-4 | $C_5H_{10}O_2$ | 869 | 1753 | 1733 ± 13(119) | 0.071 |
| cheesy | Heptanoic acid | 111-14-8 | $C_7H_{14}O_2$ | 885 | 1966.1 | 1950 ± 15(90) | 0.094 |
| cheesy | Butanoic acid | 107-92-6 | $C_4H_8O_2$ | 966 | 1644 | 1625 ± 12(290) | 0.237 |
| cheesy | Butanoic acid, 3-methyl- | 503-74-2 | $C_5H_{10}O_2$ | 857 | 1682.5 | 1666 ± 11(258) | 2.893 |
| caramel | 2-Propanone, 1-hydroxy- | 116-09-6 | $C_3H_6O_2$ | 829 | 1298.7 | 1303 ± 12(62) | 0.119 |

***Figure 5. Details of analytes that comprise the dairy aroma component of the chocolate sample.***




| Odor | Analyte | CAS | Formula | Sim | RI | Library RI | Area % |
|---|---|---|---|---|---|---|---|
| fruity | 3-methyl-butanal | 590-86-3 | $C_5H_{10}O$ | 900 | 920.5 | 918 ± 7(202) | 0.374 |
| fruity | butanoic acid, ethyl ester | 105-54-4 | $C_6H_{12}O_2$ | 813 | 1039.6 | 1035 ± 8(251) | 0.069 |
| fruity | 2-pentyl-furan | 3777-69-3 | $C_9H_{14}O$ | 832 | 1232.4 | 1231 ± 9(167) | 0.115 |
| fruity | hexanoic acid, ethyl ester | 123-66-0 | $C_8H_{16}O_2$ | 788 | 1235.1 | 1233 ± 9(275) | 0.059 |
| fruity | ethyl lactate | 97-64-3 | $C_5H_{10}O_3$ | 847 | 1343.9 | 1347 ± 9(77) | 0.018 |
| fruity | 2-nonanone | 821-55-6 | $C_9H_{18}O$ | 914 | 1388 | 1390 ± 7(127) | 0.287 |
| fruity | 2-undecanone | 112-12-9 | $C_{11}H_{22}O$ | 729 | 1597.8 | 1598 ± 6(94) | 0.044 |
| fruity | 3-isoamyl-2,5-dimethylpyrazine | 18433-98-2 | $C_{11}H_{18}N_2$ | 826 | 1653.4 | 1666 ± 0(3) | 0.006 |
| fruity | ethyl acetate | 141-78-6 | $C_4H_8O_2$ | 946 | 900.1 | 888 ± 8(234) | 0.040 |
| fruity | 2-butanone | 78-93-3 | $C_4H_8O$ | 854 | 908.5 | 907 ± 11(109) | 0.042 |
| fruity | n-propyl acetate | 109-60-4 | $C_5H_{10}O_2$ | 893 | 978 | 973 ± 11(75) | 0.345 |
| banana | Isoamyl acetate | 123-92-2 | $C_7H_{14}O_2$ | 830 | 1125.2 | 1122 ± 7(168) | 0.775 |
| cherry | benzaldehyde | 100-52-7 | $C_7H_6O$ | 952 | 1523.9 | 1520 ± 14(471) | 0.538 |
| citrus | 3-carene | 13466-78-9 | $C_{10}H_{16}$ | 789 | 1143.3 | 1147 ± 7(192) | 0.027 |
| citrus | limonene | 138-86-3 | $C_{10}H_{16}$ | 938 | 1192.7 | 1200 ± 7(759) | 10.407 |
| citrus | 2-heptanol | 543-49-7 | $C_7H_{16}O$ | 932 | 1319.8 | 1320 ± 9(96) | 0.611 |
| coconut | delta-decalactone | 705-86-2 | $C_{10}H_{18}O_2$ | 762 | 2195.9 | 2194 ± 15(73) | 0.011 |

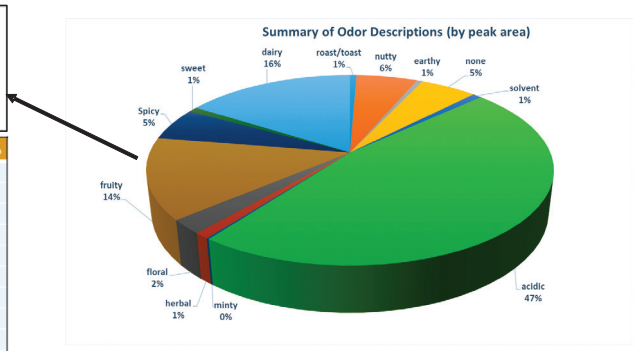



***Figure 6. Details of analytes that comprise the fruity odor component of the chocolate sample.***

Specific aromas can be connected with specific chemicals through this type of analysis and a more complete chemical characterization and understanding of your sample is possible.

## Conclusion

In this work, we aimed to provide a comprehensive characterization of the identified volatile and semi-volatile components of a chocolate sample. The *Pegasus* BT is well suited for this task by efficiently separating and confidently identifying analytes in the samples. With identifications, aroma characteristics were determined and the overall chemical aroma profile was calculated with deconvoluted TIC peak areas. GC-MS helps you learn more about your samples of interest.

## References

[1]http://www.thegoodscentscompany.com/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