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## *Instrument: Pegasus® BT 4D*

# Workflow for the Assessment of Key Aroma Compounds of Pumpernickel Bread Variations

## *An Analytical Workflow Combining GCxGC-TOFMS Analysis and Automated Statistical Data Processing*

Key Words: GCxGC, TOFMS, VOCs, Flavor and Food, Aroma, Bread, Pumpernickel, Statistical Data Analysis, ChromaTOF® Tile

## *Introduction*

Pumpernickel is a special type of sour dough bread made traditionally of rye. It originates from the Westphalia region of Germany; it is very dark and has a dense and coarse structure due to its baking process, which is slow and involves steam-cooking conducted under moderate conditions for a relatively long time. A variety of flavors develop during this process due to the formation of a number of Maillard reaction products including some key aroma compounds[1]. Consumers enjoy the sweet taste and intense aroma, typically described as brown, caramel, and malty, but its nutritional value is also appreciated, such as its relatively low glycemic index. Variations to the recipe have an impact on the final product including its taste and aroma profile. In-depth understanding of the effects of such alterations can help producers to tailor the creation of products and their flavors and aromas efficiently and according to consumer preferences.

This application note describes a complete analytical workflow for the analysis of pumpernickel bread varieties, using solvent-assisted flavor evaporation (SAFE), a comprehensive two-dimensional gas chromatography (GCxGC) system coupled to time-of-flight mass spectrometry (TOFMS), and a powerful statistical data processing software. The use of the LECO *Pegasus* BT4D GCxGC-TOFMS system provided excellent separation power, dynamic range, and sensitivity, allowing detection of a high number of aroma-relevant compounds. The ability to collect data at high acquisition rates over a wide mass range also enabled peak finding with mass spectral (MS) and retention index (RI) library searching to be performed quickly via automated deconvolution.

Interpretation of the sample set was performed using *ChromaTOF* Tile Software, enabling an efficient, supervised statistical analysis via production of a composite sample set feature table, where trends, patterns, and significant differences between features of interest in the samples were observed.

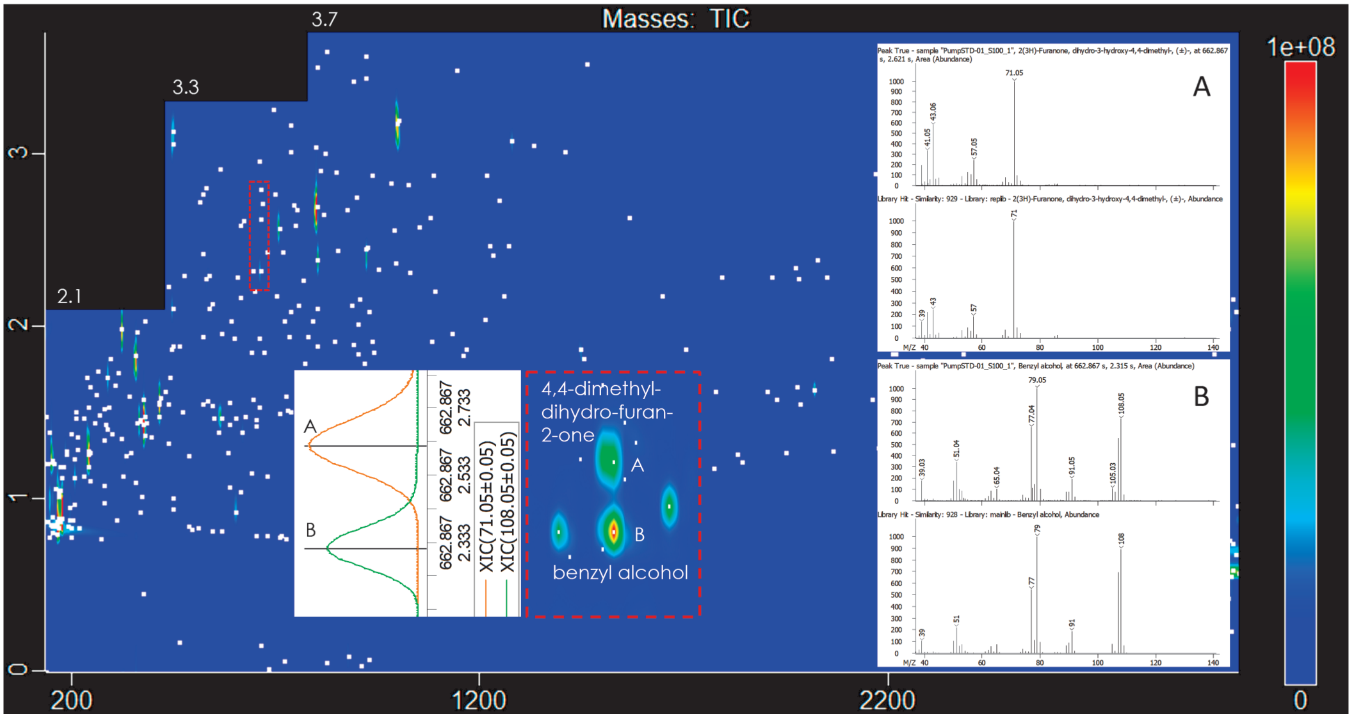


***Figure 1:** Total ion current (TIC) contour plot of a pumpernickel extract.*

## Experimental

Three pumpernickel bread varieties were extracted with methylene chloride prior to solvent-assisted flavor evaporation (SAFE)[1] and were analyzed in triplicate according to the conditions stated in Table 1. A n-alkane standard (C7-C30) was analyzed for calculation of linear retention indices (RIs).

**Table 1: Sampling and analysis parameters.**

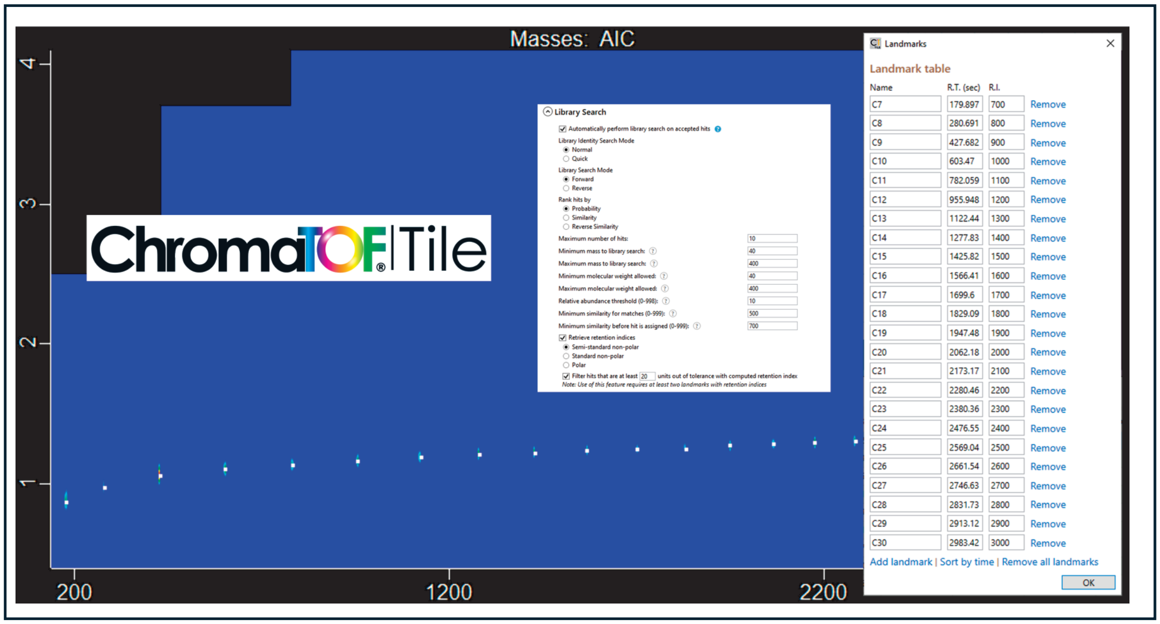
| **GC** | **Agilent 7890** |
|---|---|
| Injector | 1 $\mu$L split mode (1:100), 250 °C |
| Carrier Gas | He 1.4 mL/min, constant flow |
| Column | $D_1$: Rxi-5MS 30 m x 0.25 mm i.d. x 0.25 $\mu$m coating<br>$D_2$: Rxi-17MS 0.9 m x 0.25 mm i.d. x 0.25 $\mu$m coating |
| Oven Temperature Program | 40 °C (1 min), 5 °C/min to 280 °C; 20 °C/min to 300 °C (1 min) |
| Secondary Oven | +11 °C (relative to the main oven temperature) |
| Modulator | +15 °C (relative to the secondary oven temperature) |
| Modulation Time | 2.1 s (start - 431 s); 3.3 (432 s - 778 s); 3.7 s (779 s – end) |
| Transfer Line | 330 °C |
| **MS** | **LECO *Pegasus* BT4D** |
| Ion Source Temp | 250 °C |
| Mass Range | 40–400 m/z |
| Acquisition Rate | 200 spectra/s |

## Results and Discussion

Figure 1 shows a chromatogram from one of the pumpernickel bread extracts. The complexity of these types of samples is apparent with a high number of peaks visible in the total ion chromatogram (TIC). One of the benefits of coupling GCxGC with TOFMS detection is that highly comprehensive non-target data is acquired due to both the two modes of separation and the extremely fast data acquisition rates over the whole mass range. The increased sensitivity in comparison to one-dimensional GC is due to the band focusing during the process of thermal modulation. For this analysis, a variable modulation period (VMP) was applied and is indicated by the steps on the left upper part of chromatogram, with the time of the modulation period ($P_M$) reported for every section. The use of a VMP allows us to optimize and fine-tune the separation, preserving the first column ($D_1$) chromatographic resolution for critical pairs.

Highlighted is a retention window indicating an area of the chromatogram where two components with the same first dimension retention time (RT1) elute; however, these two components were clearly separated in the second dimension ($D_2$). The mass spectral information and RIs were used to identify the 2D resolved compounds (marked with A and B). A spectral comparison performed using the NIST MS library revealed a similarity score of 929/1000 for 4,4-dimethyl-dihydro-furan-2-one and 928/1000 for benzyl alcohol for A and B, respectively.

Typically, the alignment and comparison of multiple chromatograms displays a major challenge in GCxGC-MS data processing. Here, it was possible to find similarities and differences among the analyzed pumpernickel bread extracts in a straightforward manner utilizing *ChromaTOF* Tile. This processing software platform is based on the tile-based Fisher ratio approach and can compare multiple GCxGC-TOFMS data sets in a fast and user-friendly way. Statistically significant differences can be then highlighted, reducing the time required to locate chromatographic differences between the sample sets. For improved compound identification, the software enables the implementation of RI calculation easily as displayed in Figure 2.



Library Search

☑ Automatically perform library search on accepted hits

Library Identity Search Mode
● Normal
○ Quick

Library Search Mode
● Forward
○ Reverse

Rank hits by
● Probability
○ Similarity
○ Reverse Similarity

Maximum number of hits: 10
Minimum mass to library search: 40
Maximum mass to library search: 400
Minimum molecular weight allowed: 40
Maximum molecular weight allowed: 400
Relative abundance threshold (0-998): 10
Minimum similarity for matches (0-999): 500
Minimum similarity before hit is assigned (0-999): 700

☑ Retrieve retention indices
● Semi-standard non-polar
○ Standard non-polar
○ Polar

☑ Filter hits that are at least 20 units out of tolerance with computed retention index
*Note: Use of this feature requires at least two landmarks with retention indices*

Landmark table

| Name | R.T. (sec) | R.I. | |
|---|---|---|---|
| C7 | 179.897 | 700 | Remove |
| C8 | 280.691 | 800 | Remove |
| C9 | 427.682 | 900 | Remove |
| C10 | 603.47 | 1000 | Remove |
| C11 | 782.059 | 1100 | Remove |
| C12 | 955.948 | 1200 | Remove |
| C13 | 1122.44 | 1300 | Remove |
| C14 | 1277.83 | 1400 | Remove |
| C15 | 1425.82 | 1500 | Remove |
| C16 | 1566.41 | 1600 | Remove |
| C17 | 1699.6 | 1700 | Remove |
| C18 | 1829.09 | 1800 | Remove |
| C19 | 1947.48 | 1900 | Remove |
| C20 | 2062.18 | 2000 | Remove |
| C21 | 2173.17 | 2100 | Remove |
| C22 | 2280.46 | 2200 | Remove |
| C23 | 2380.36 | 2300 | Remove |
| C24 | 2476.55 | 2400 | Remove |
| C25 | 2569.04 | 2500 | Remove |
| C26 | 2661.54 | 2600 | Remove |
| C27 | 2746.63 | 2700 | Remove |
| C28 | 2831.73 | 2800 | Remove |
| C29 | 2913.12 | 2900 | Remove |
| C30 | 2983.42 | 3000 | Remove |

***Figure 2: Implementation of RI in ChromaTOF Tile.***

Principal Component Analysis (PCA) was used to display clustering of samples based on their overall similarity and trends. Figure 3 shows the PCA scores plot obtained with the three clusters according to their aroma profiles linked to the modifications of the recipes.

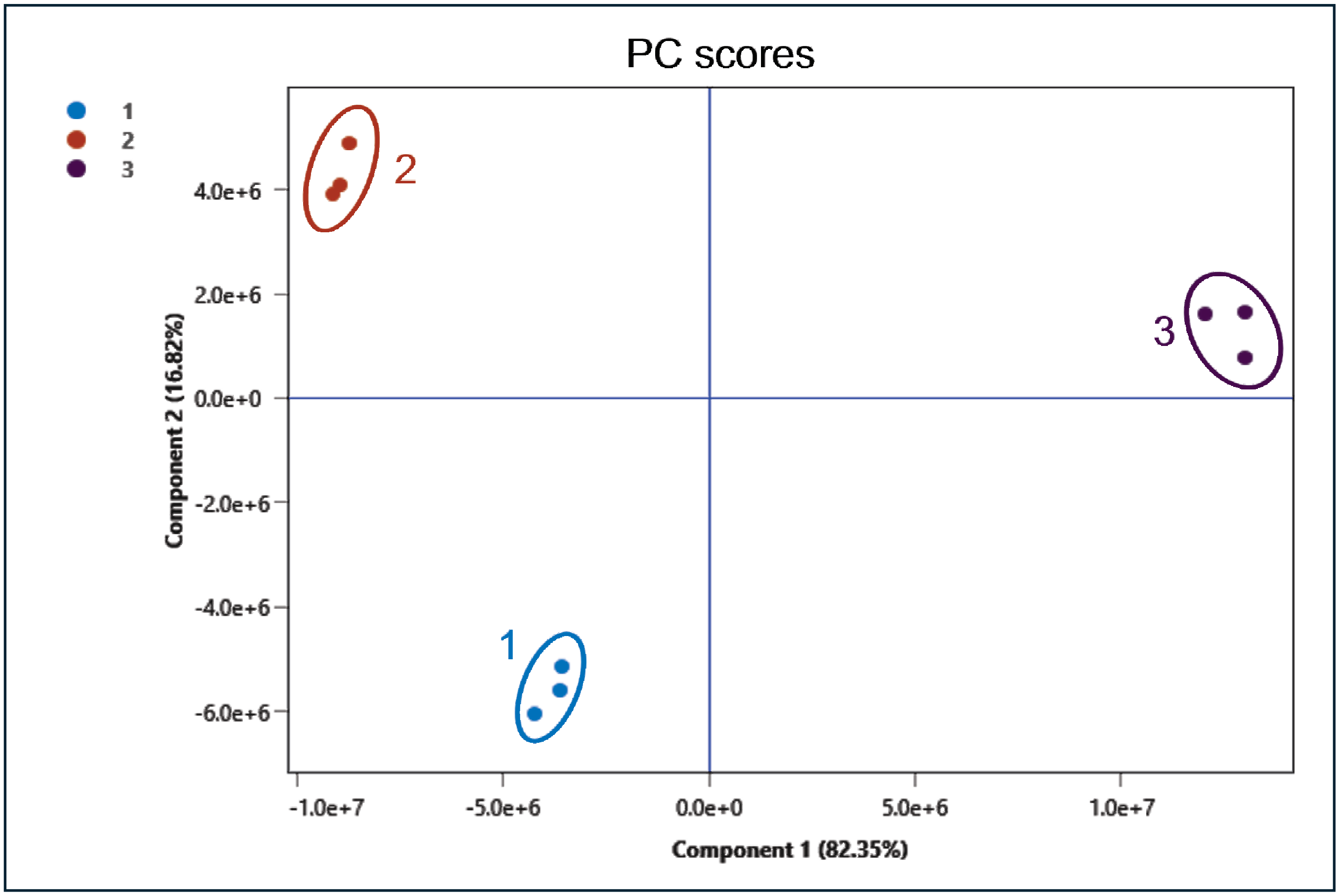
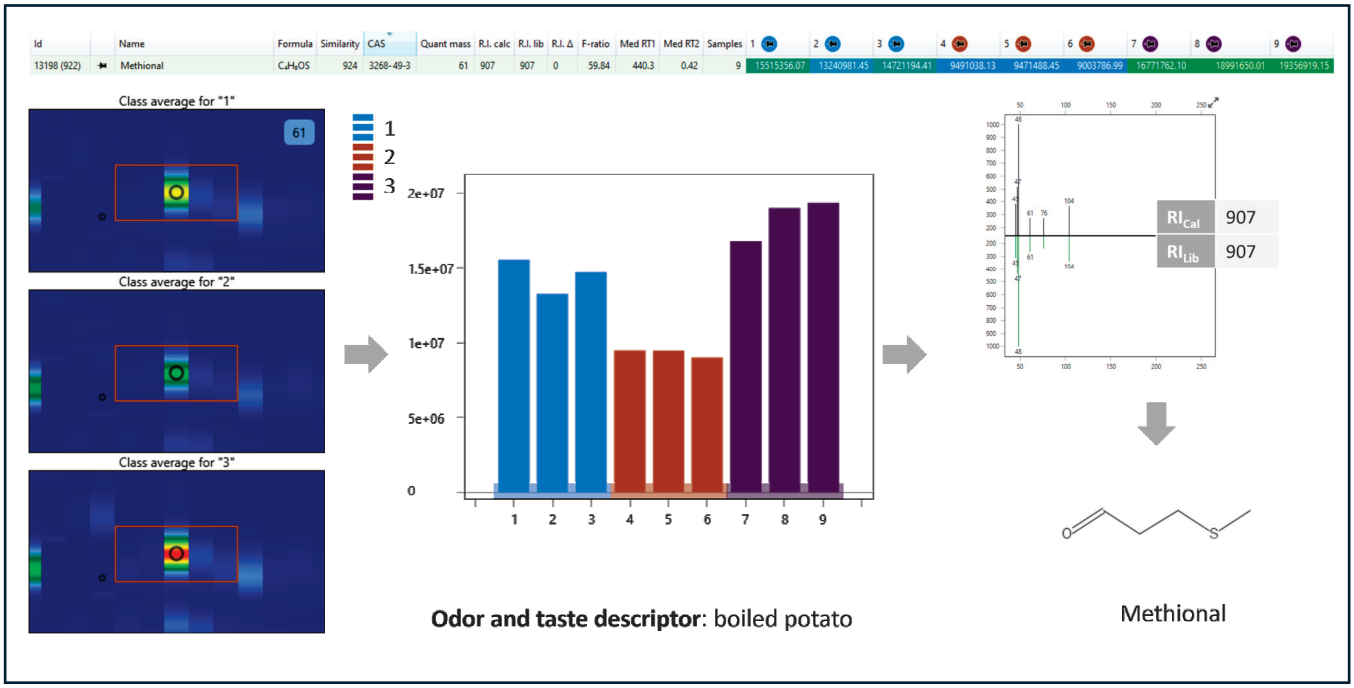


***Figure 3: Principal Component Analysis (PCA) scores plot displaying clustering of the Pumpernickel bread extracts according to their aroma-type and recipe.***

The distribution of individual features can be explored through the sample set employing different visualization tools e.g., a classical bar chart display, contour plot (class average or individual samples), or heat map as part of the chemical feature table.

Figure 4 shows the trend of one of the features with a high Fisher ratio indicating significant differences between sample classes. The observed spectrum matched to methional (CAS: 3268-49-3) in the NIST library database with a similarity score of 925. This identification was further supported with RI information. The calculated RI for this peak was 907, showing perfect agreement with the library RI value (= 907). Methional was detected in all three samples; however, the highest abundance was detected in sample 3 and 2. Methional is an odor-active compound, and it is known for its boiled potato smell.

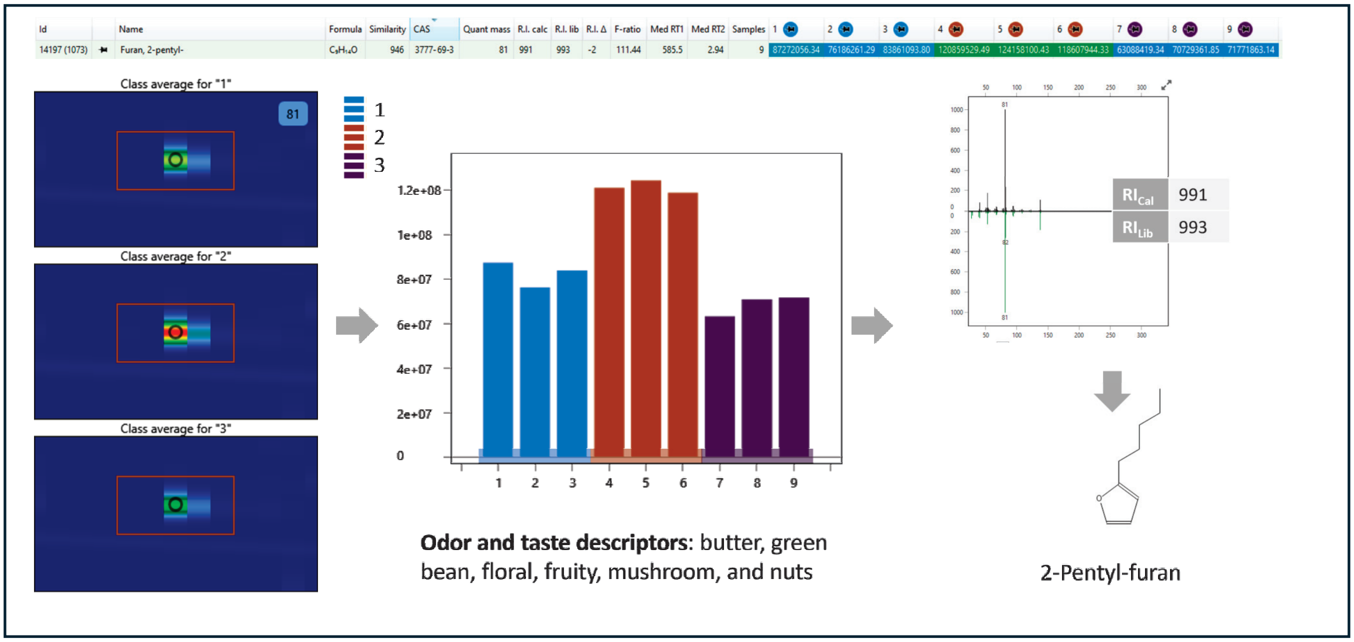


| Id | | Name | Formula | Similarity | CAS | Quant mass | R.I. calc | R.I. lib | R.I. Δ | F-ratio | Med RT1 | Med RT2 | Samples | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 13198 (922) | 📌 | Methional | $C_4H_8OS$ | 924 | 3268-49-3 | 61 | 907 | 907 | 0 | 59.84 | 440.3 | 0.42 | 9 | 15515356.07 | 13240981.45 | 14721194.41 | 9491038.13 | 9471488.45 | 9003786.99 | 16771762.10 | 18991650.01 | 19356919.15 |

**Odor and taste descriptor**: boiled potato

*Figure 4: Averaged contour plot (m/z = 61), mass spectral comparison and bar chart of methional (CAS: 3268-49-3, RIΔ = 0) in all analyzed pumpernickel bread extracts.*

The prominence of another statistically meaningful compound and its variability among the samples is displayed in Figure 5. The observed MS spectrum was matched to 2-pentyl furan (CAS: 3777-69-3) in the NIST library database with a similarity score of 946/1000. This identification was further supported with RI information. The calculated RI for this peak was 991, showing good agreement with the library RI value of 993. A higher occurrence, as indicated in the bar chart, was observed in the extract number 2. The odor and taste descriptors of 2-pentyl furan include the following: butter, green bean, floral, fruity, mushroom, and nuts.

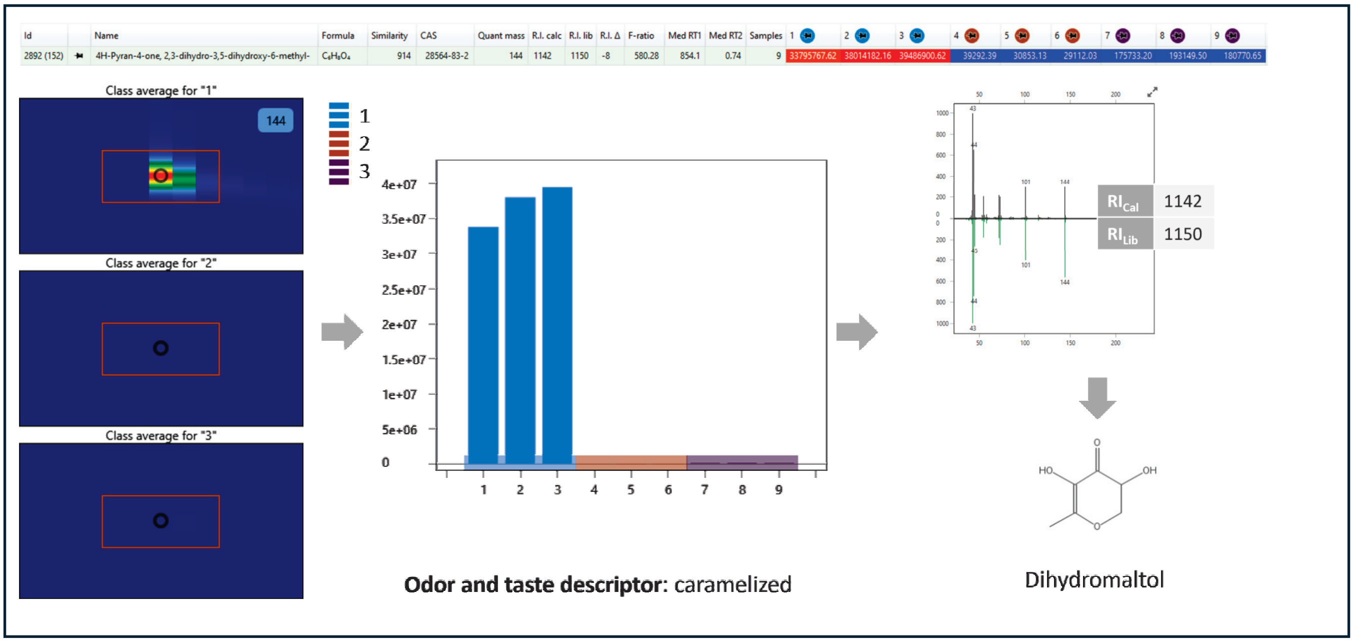


| Id | | Name | Formula | Similarity | CAS | Quant mass | R.I. calc | R.I. lib | R.I. Δ | F-ratio | Med RT1 | Med RT2 | Samples | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 14197 (1073) | 📌 | Furan, 2-pentyl- | $C_9H_{14}O$ | 946 | 3777-69-3 | 81 | 991 | 993 | -2 | 111.44 | 585.5 | 2.94 | 9 | 87272056.34 | 76186261.29 | 83861093.80 | 120859529.49 | 124158100.43 | 118607944.33 | 63088419.34 | 70729361.85 | 71771863.14 |

**Odor and taste descriptors**: butter, green bean, floral, fruity, mushroom, and nuts

*Figure 5: Averaged contour plot (m/z: 81), mass spectral comparison and bar chart of 2-pentyl-furan (CAS: 3777-69-3, RIΔ = 2) in all analyzed pumpernickel bread extracts.*

Figure 6 shows the trend of a compound identified as 2,3-dihydro-3,5- dihydroxy-6-methyl-4Hpyran-4-one (CAS: 28564-83-2) also known as dihydromaltol (DHM). The similarity score in the NIST library database was 914/1000 and the RI difference (RIΔ) between the calculated and the library RI was 8. The presence of this caramelized-smelling compound was relatively low in the extracts number 2 and 3; however, very high concentrations in the sample number 1 were found.



| Id | | Name | Formula | Similarity | CAS | Quant mass | R.I. calc | R.I. lib | R.I. Δ | F-ratio | Med RT1 | Med RT2 | Samples | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2892 (152) | | 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- | $C_6H_8O_4$ | 914 | 28564-83-2 | 144 | 1142 | 1150 | -8 | 580.28 | 854.1 | 0.74 | 9 | 33795767.62 | 38014182.16 | 39486900.62 | 39292.39 | 30853.13 | 29112.03 | 175733.20 | 193149.50 | 180770.65 |

***Figure 6: Averaged contour plot (m/z = 144) mass spectral comparison and bar chart of dihydromaltol (CAS: 28564-83-2, RIΔ = 8) in all analyzed pumpernickel bread extracts.***

Understanding similarities and difference in the chemical composition and the associated aroma profiles of pumpernickel bread may help to achieve the production of foods with sensory characteristics more appreciated by consumers.

## Conclusion

GCxGC-TOFMS combined with *ChromaTOF* Tile was used to analyze the aroma profile of pumpernickel bread of different recipes. The analytical workflow based on the LECO`s *Pegasus* BT4D GCxGC-TOFMS technology generates rich, high-quality data. The *ChromaTOF* Tile Software is particularly suitable for non-targeted data analysis when comparing different classes or groups of samples. Compounds of interest were identified based on comparison of mass spectral information within *ChromaTOF* Tile and NIST MS libraries and retention indices calculations. The distribution of differentiating compounds such as methional, 2-pentyl-furan and dihydromaltol can be easily visualized using tools such as a bar chart. The proposed workflow can be utilized to explore aroma profiles and follow individual analyte trends through the analyzed samples as well as overall sample trends within the tested sample set. Such studies may have a number of important implications for food production and reformulation practice, since better knowledge of the volatile compounds produced along the Maillard reaction may assist the creation and modification of foods with specific aroma characteristics.

## Authors

This application was developed in collaboration by LECO Europe EATC, Germany and The Faculty of Food Science & Nutrition, Poznan University of Life Sciences, Poznan, Poland (Prof. M. A. Majcher).

## References

[1]M. A: Majcher et al., Formation of Key Aroma Compounds during Preparation of Pumpernickel Bread. J. Agric. Food Chem. 2020, 68, 38, 10352–10360.

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