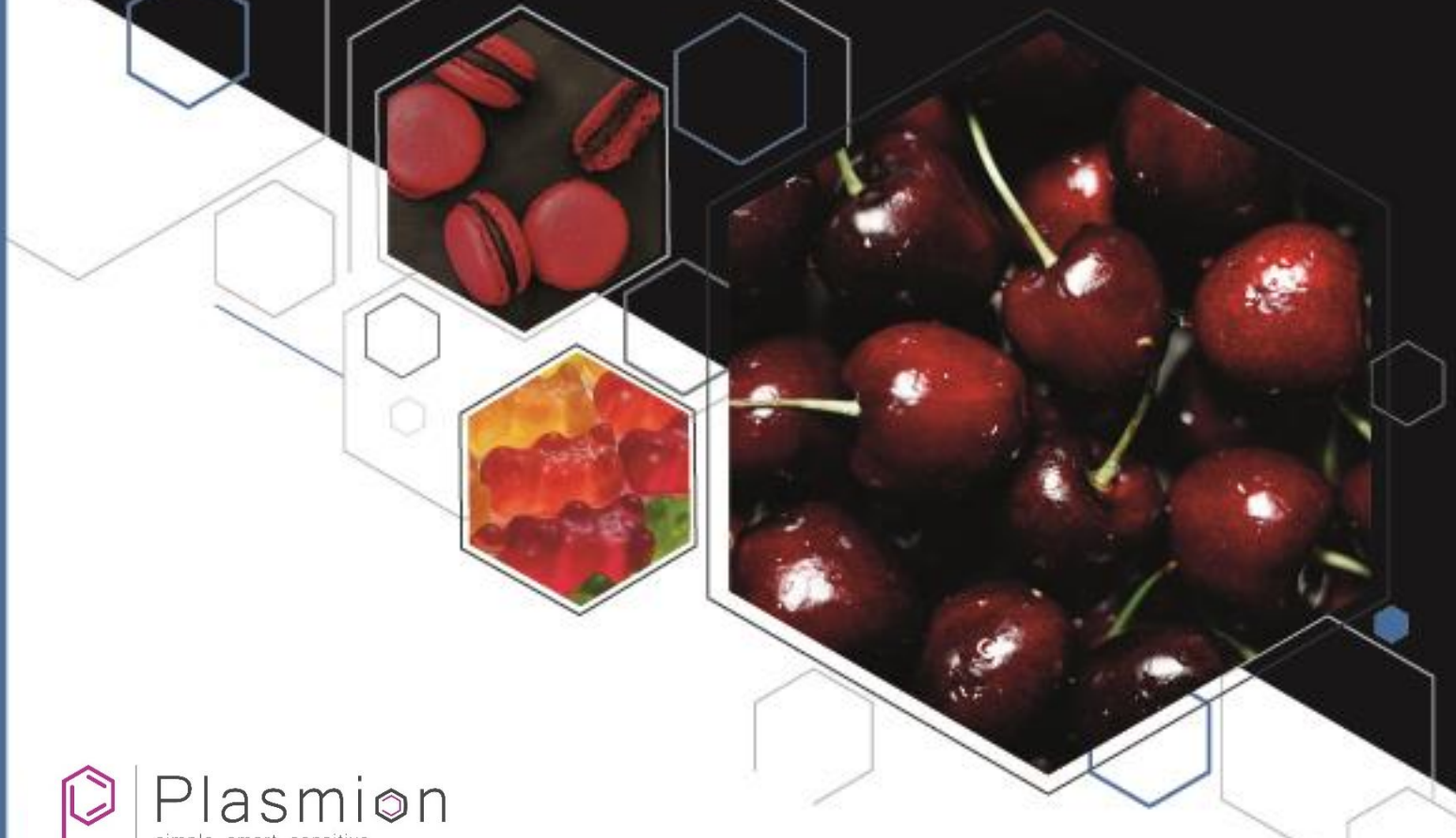
HaVoc
sensory system

Characterization of cherry-type flavorings based on volatile compounds by means of the HaVoc sensory system



Plasmion
simple smart sensitive

Characterization of cherry-type flavorings based on volatile compounds by means of the HaVoc sensory system

Introduction

Flavorings are very popular as they provide an extra taste to foods and beverages such as pastries, ice cream, sweets, coffee, or tea. Additionally, cosmetics such as creams or toothpastes are often scented with respective flavorings. Therefore, flavorings are of special interest, particularly for the food and cosmetics industry.

However, the use of flavorings has sparked controversial discussions, mainly revolving about two themes: naturalness and consistency. Since it is legally allowed to declare flavors as 'natural' even if they are not directly derived from a natural product, such as cherry, but obtained by enzymatic or microbiological processes. As a result, many producers claim that their flavors are of natural origin, even if they are not [1]. Furthermore, consumer awareness of taste, smell, and ingredients is a growing topic and consumers are quite sensitive to differences in taste or smell once they become accustomed to a specific product aroma [2]. This increases the pressure on producers in terms of reproducibility and quality standards.

The real-time analysis of flavorings and aroma patterns remains a significant challenge. In many companies, especially in the food and fragrance industry, the human nose is still the preferred method. However, this method is very expensive due to training required for panelists and moreover is prone to subjectivity, resulting in drawbacks, particularly in terms of comparability and reproducibility. From an analytical standpoint, gas chromatography (GC) coupled with mass spectrometry (MS) is the most common method for flavoring analysis. However, this analysis technique is time consuming (approx. 5-30 minutes per analysis, depending on the instrument and program) and is unsuitable for real-time analysis.

In this application note, we will introduce the capabilities of our new sensor system (HaVoc) for real-time analysis of volatile organic compounds (VOCs). This system is based on mass spectrometry and delivers real-time results of laboratory-grade quality. The capabilities of HaVoc will be demonstrated through cherry aroma analysis. For this purpose, we analyzed 11 different cherry flavorings from six different manufacturers, encompassing common cherry flavorings as well as sweet cherry, sour cherry, morello cherry, wild cherry and amarena cherry (Table 1).

The overall objective of this study is to showcase the capabilities of the HaVoc system in terms of categorization, comparison, and chemical composition of flavorings. Therefore, the following hypotheses for the HaVoc system have been investigated.

Producer	Flavoring type	Abbreviation	Additional Declaration
#1	Amarena	#1-AM	Nature identical flavoring
	Morello	#1-MO	Nature identical flavoring
	Sweet	#1-SW	Nature identical flavoring
	Wild	#1-WI	Nature identical flavoring
#2	Amarena	#2-AM	Natural and nature identical flavorings
	Cherry	#2-CH	Natural and nature identical flavorings
#3	Sour	#3-SO	n.A.
	Sweet	#3-SW	n.A.
#4	Cherry	#4-CH	Natural flavoring
#5	Cherry	#5-CH	Natural flavoring
#6	Cherry	#6-CH	Natural cherry flavoring

Table 1 – Overview of the producers and cherry type flavorings.

©2023 Plasmion GmbH

All rights reserved

Am Mittleren Moos 48
D-86167 Augsburg
www.plasmion.de

T: +49 (0) 821 20713355
E: info@plasmion.de

Clustering and comparison:

- H1 – HaVoc can distinguish and cluster different cherry-type flavorings from one producer as well as different producers based on one supposing equally categorized "cherry" flavoring.

Human perception vs. technology-based assessment:

- H2 – HaVoc can be used to mimic or validate the odor perception of a human sensory panel.

Chemical composition:

- H3 – HaVoc can identify similarities and differences of different cherry-type flavorings with respect to their ingredients.

Analysis & Data

Clustering and comparison – H1:

To validate that HaVoc can cluster and compare different cherry-type flavorings from one producer, the volatile fingerprint of 4 different flavorings (Amarena = AM, Morello = MO, Sweet = SW, Wild = WI) from producer #1 were measured and analyzed. Similarly, to proof that HaVoc can also distinguish and cluster different producers based on one assumed equally categorized flavoring, the "cherry" flavorings from four different producers (#2, #4, #5, #6) were investigated in a second analysis. For each analysis, 3 samples (replicates) of each flavoring were prepared with 10 mg liquid solution in a 20 mL headspace vial. The HaVoc system was equipped with a PAL autosampler (CTC-Analytics). The analysis was performed by collecting 2 mL headspace in a headspace syringe and injecting them into an injection port. Alternatively, another viable option would be to place the headspace vial directly in front of the HaVoc inlet system and 'sniff' the volatile profile. The derived volatile fingerprints (average spectra across the 3 replicates) of the 4 analyzed cherry-type flavorings from producer #1 are depicted in Figure 1, and those of the 4 analyzed cherry flavorings from different producers are depicted in Figure 2.

The visual comparison of the volatile fingerprints already enables us to identify similarities and differences based on presence and intensity of different signals (peaks). However, to validate this impression, statistical methods have been employed to draw data-driven conclusions. For both the flavoring comparison and the producer comparison, a principal component analysis (PCA) was performed. In the PCAs, the spectral raw data were corrected by their sample weight and normalized by the sum of the corresponding spectrum. The results of each PCA are depicted in a scores plot and a loadings plot (Figure 1 and 4).

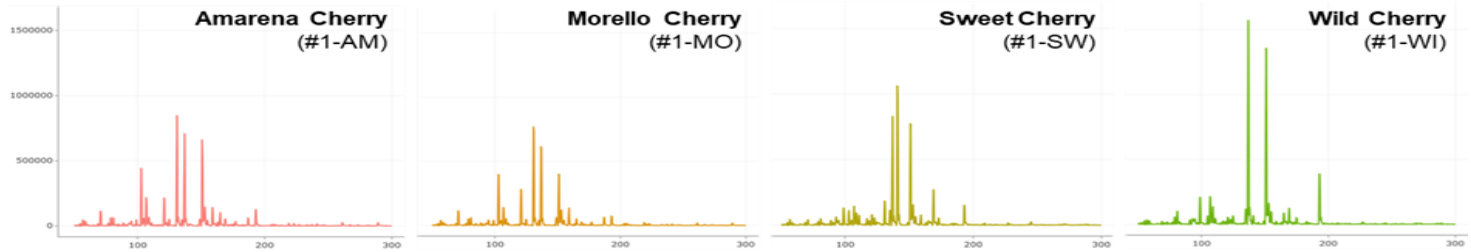


*Figure 1 – Volatile fingerprint of **different cherry-type flavorings** from producer #1.*

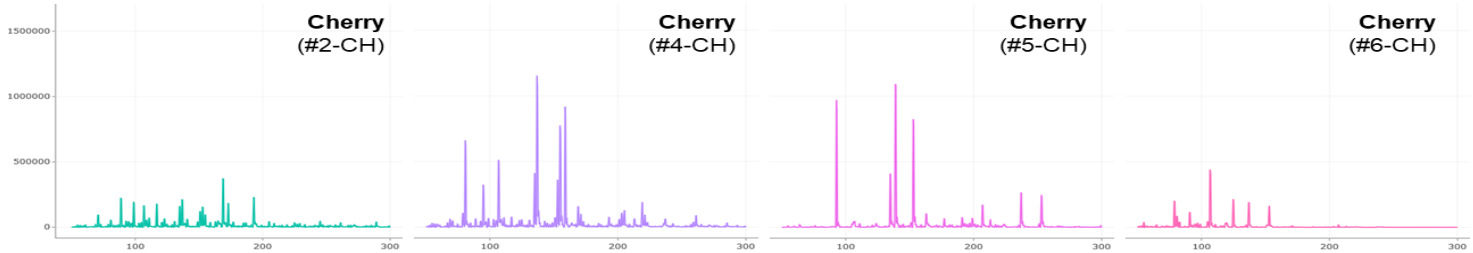


*Figure 2 – Volatile fingerprint of the cherry flavorings from **different producers**.*

©2023 Plasmion GmbH

All rights reserved

Am Mittleren Moos 48
D-86167 Augsburg
www.plasmion.de

T: +49 (0) 821 20713355
E: info@plasmion.de

The scores plot demonstrates the clustering of the different flavorings, while the loadings plot indicates the signals responsible for the clustering and categorization. Signals that are farther from the center of the loadings plot have a greater impact on the clustering, and their direction corresponds to the separation observed in the scores plot. In the first analysis, the scores plot reveals that the amarena and morello flavorings cluster closely together, while the sweet cherry and wild cherry flavorings are clearly separated. The cluster formed by amarena and morello suggests that these two flavorings have similar spectra and therefore a similar composition. According to the loadings plot, this common cluster of amarena and morello flavorings was induced by the signals 131, 103, and 121. The sweet cherry flavoring is distinctly separated due to signal 141, while the wild cherry flavorings are distinguished by signals 151 and 137.

In the analysis of the producers, the scores plot shows that the cherry flavorings from producer #5 and producer #6 are more widely separated than the samples from producer #2 and producer #4. This suggests that the volatile fingerprints of the flavorings from producer #2 and producer #4 are more similar than the volatile fingerprints from producer #5 and producer #6. The loadings plot reveals that the signals

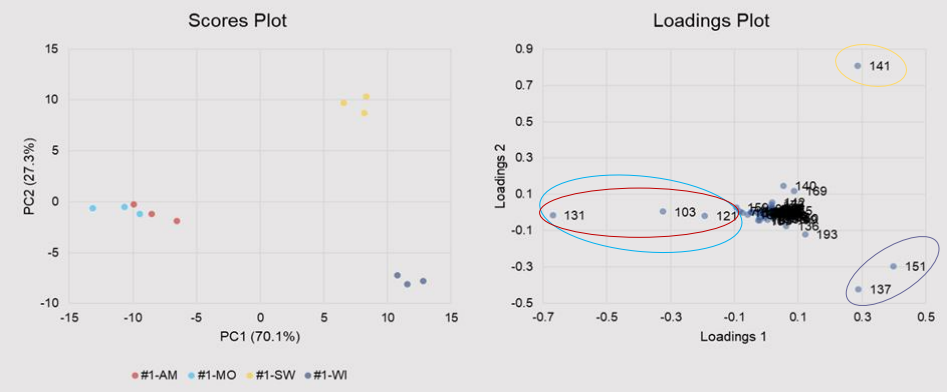


*Figure 3 – Scores and loadings plot (PCA) of the flavorings **amarena** cherry, **morello** cherry, **sweet** cherry, and **wild cherry** from **producer #1**.*

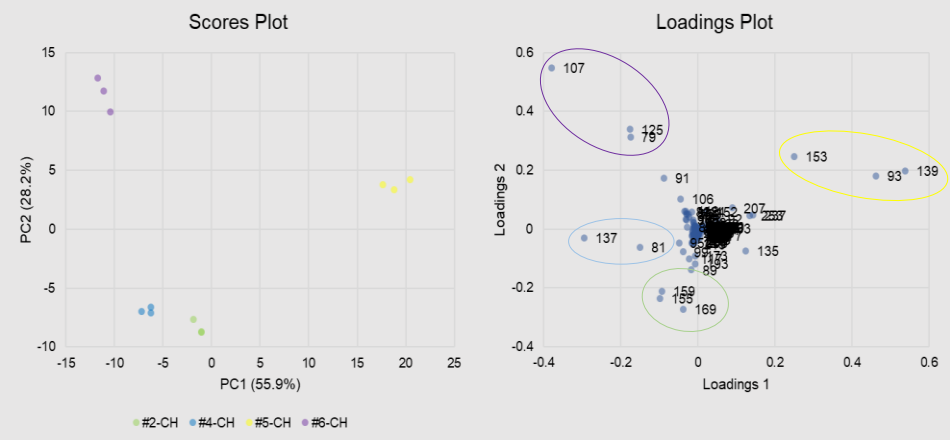


*Figure 4 – Scores and loadings plot (PCA) of the **cherry flavorings** from **different producers**.*

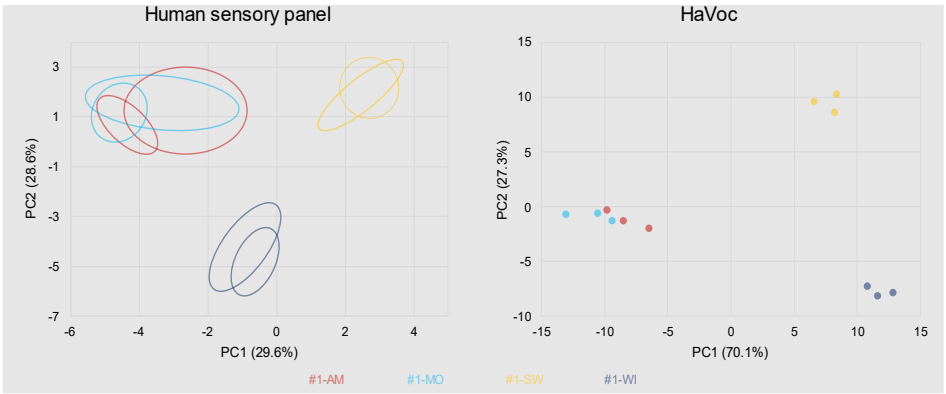


*Figure 5 – Differentiation of cherry-type flavorings by means of a **human sensory** panel **and** the **HaVoc** system.*

169, 155, and 159 are responsible for the separation from producer #2, while the separation of producer #4 is attributed to the signals 137 and 81. Producer #5 is clearly separated by signals 139, 93, and 153, and producer #6 is distinguished by signals 107, 125, and 79.

Human perception vs. technology-based assessment – H2

To assess whether HaVoc can mimic or validate the odor perception of a human sensory panel, the four flavorings (amarena, morello, sweet, and wild cherry) from

©2023 Plasmion GmbH

All rights reserved

Am Mittleren Moos 48
D-86167 Augsburg
www.plasmion.de

T: +49 (0) 821 20713355
E: info@plasmion.de

producer #1 were evaluated by both a human sensory panel and the HaVoc system. The analysis of the human sensory panel was conducted using 2 samples (replicates) of each flavoring, with 80 mg of liquid solution in a headspace vial. All samples were assigned a randomly selected three-digit numeric code. The 11 panelists were instructed to smell the samples and to position them on a DIN A3 sheet of paper based on their perceived similarity, with similar samples placed close together and dissimilar samples far apart. The panelists were informed that the samples could be categorized into four groups of different cherry-type flavorings. The positions of the samples on the paper were recorded as X- and Y-coordinates. The data was subjected to a PCA to assess the clustering and discrimination of the different cherry-type flavorings.

The results of the human sensory panel were compared with the HaVoc analysis results, including the respective PCA scores plot (Figure 3), of the four different cherry-type flavorings from producer #1, which were previously analyzed to confirm H1.

The PCA clustering performed by the human sensory panel reveals that the panelists were able to distinguish two out of four flavorings (Figure 5). The flavorings sweet cherry and wild cherry could be separated by the human sensory panel, while the flavorings amarena and morello exhibited significant overlap in their respective clusters. The HaVoc analysis yielded similar results, successfully discriminating the sweet cherry and wild cherry flavorings, but showing close proximity between the amarena and morello flavorings. This demonstrates that HaVoc is a data-driven solution that can potentially support and validate results obtained from a human sensory panel.

However, HaVoc might even outperform the human sensory panel. To compare the separation capabilities of the two analyses, the variances of the PCAs (expressed as percentage values on the scores plots axis labels) were evaluated. A higher variance indicates a better separation.

While the total variance obtained by the human sensory panel is only 58.2%, the corresponding value for HaVoc is 97.4% (70.1% + 27.3%). This suggests that, for the conducted analyses, the results obtained from the HaVoc system are more reliable in terms of separation capabilities compared to the results obtained by the human sensory panel. In conclusion, we can confirm H2 that the HaVoc system can be used to mimic or validate the odor perception of a human sensory panel and potentially surpass it.

Chemical composition – H3

To validate HaVoc's ability to identify the chemical composition of the flavorings, the spectra of the amarena and sweet cherry flavorings from producer #1 were recorded and compared to the listed ingredients. Similar to the procedure for H1, 3 samples (replicates) of each flavoring were prepared with 10 mg liquid flavoring in a 20 mL headspace vial. The spectra were recorded using the HaVoc system equipped with a PAL autosampler (CTC-Analytics). The analysis was performed by collecting 2 mL headspace in a headspace syringe and injecting it into an injection port. The derived spectra of the analyzed amarena and sweet cherry flavorings from producer #1 were averaged across the 3 replicates. Afterwards, the ingredients were assigned to the corresponding signals according to literature [3]. Some ingredients (compounds) generate several signals in the spectrum, as compounds can be ionized by several ionization mechanisms at the same time (e.g. citronellol) [3]. Consequently, some compounds have been assigned to more than one signal. But there are also cases, where two compounds generate the same signal e.g., due to their similar molecular structure. Consequently, for these cases, two compounds were assigned to the same signal. The assignment of the listed compounds to the respective signals in the spectra of the amarena and

©2023 Plasmion GmbH
All rights reserved
Am Mittleren Moos 48
D-86167 Augsburg
www.plasmion.de
T: +49 (0) 821 20713355
E: info@plasmion.de

sweet cherry flavorings from producer #1 is depicted in Figure 8.

For both flavorings, the listed compounds could all be assigned to respective signals in the spectra. It became obvious, though, that both flavorings contain multiple signals that could not be assigned to any of the listed compounds. In case of #1-AM, these signals were 71, 103, 121, 159, and 193. Comparing these signals with the listed compounds for #1-SW, it becomes quite obvious that the signal 103 can most probably be identified as benzyl alcohol. In contrast, for #1-SW, the signals 99, 107, 131, 169, and 193 could not be identified based on the given list of compounds. However, using the given compound list of #1-AM leads to the conclusion, that the signals 107 and 131 can most probably be identified as benzaldehyde and ethyl 2-methylbutanoate.

To validate if the identified ingredients not only occur in the amarena and sweet cherry flavorings from producer #1 but also in the other flavorings, the spectra of the remaining flavorings were recorded as detailed above. For the comparison of the cherry-type flavorings, the signals of the identified ingredients as well as the five most intense signals (averaged results of the 3 replicates) were selected and plotted in a heat map (Figure 9).

The signals of the identified ingredients not only occur in the amarena and sweet cherry flavorings from producer #1 but also in other flavorings. Prominent signals as 137 (D-limonene/geraniol) and 151 (piperonal) appear in many of the remaining flavorings with also high intensities. The signals 153 (vanillin) and 107 (benzaldehyde) also occur in several flavorings but with less intensity. The signal 139 of anisyl alcohol and citronellol only shows a high intensity in the flavorings #5-CH and #3-SO. The signal 131 (ethyl 2-methylbutanoate) was also detected with a high intensity but only in three flavorings from producer #1, namely P1-AM, P1-MO, and P1-SW. Only a few signals with high intensity were not identified by the given ingredient list, such as the signal 135 occurring in #3-SO, #4-CH, and #5-CH, and the signal 191, which was the most intense signal in the flavoring #2-AM. However, in both cases, the signals 135 and 191 just have a low intensity in the #1-AM and #1-SW flavorings.

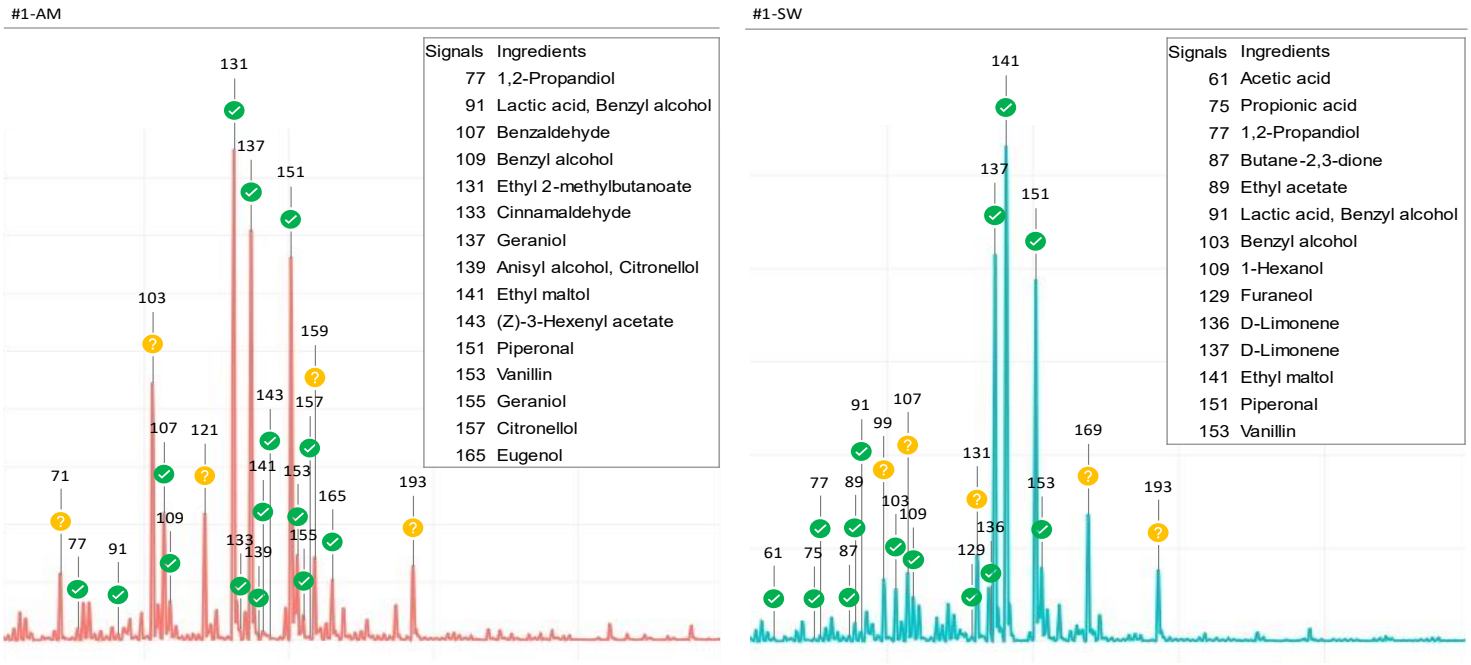


Figure 8 – Ingredients and spectra of the flavorings amarena and sweet cherry from producer #1.

©2023 Plasmion GmbH
All rights reserved
Am Mittleren Moos 48
D-86167 Augsburg
www.plasmion.de
T: +49 (0) 821 20713355
E: info@plasmion.de

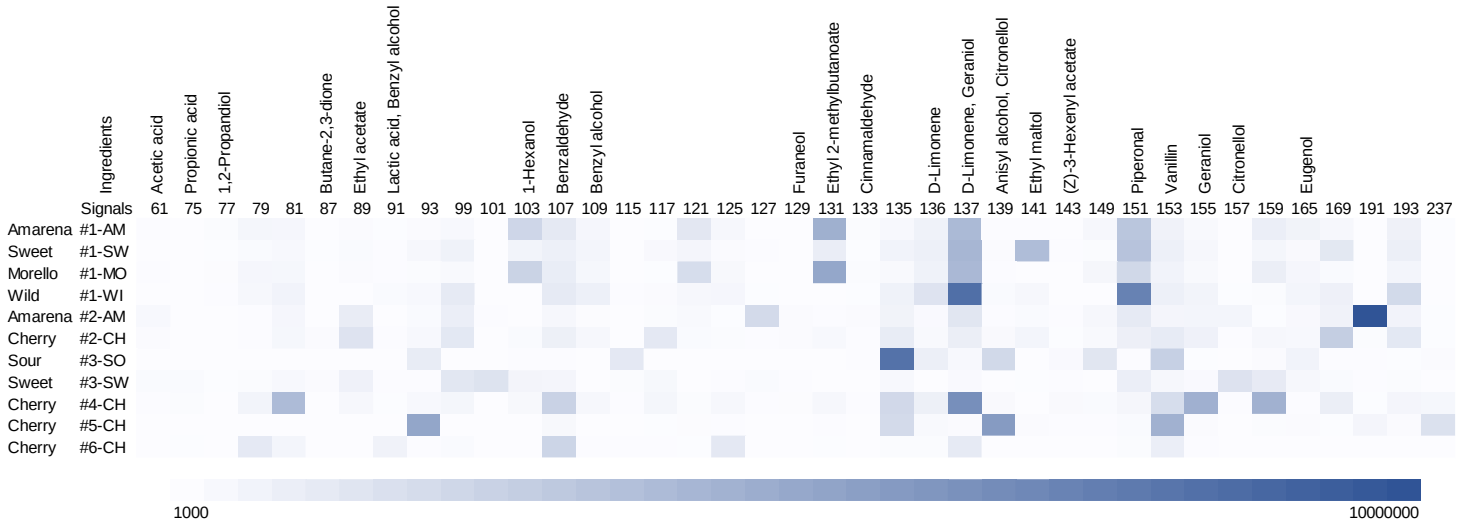


Figure 9 – Heat map of the identified ingredients plus the five most intense signals of the cherry-type flavorings

This analysis shows that the HaVoc system not only allows for the allocation of the different signals based on a reasonable list of ingredients but also enables the identification of other signals that might not even be mentioned in the list of ingredients for the respective flavorings. In the example at hand, the system was able to identify the chemical composition of the cherry-type flavorings. Through the identification of the chemical composition, most of the intense signals in the cherry-type flavorings were clarified, and only a few signals remained unidentified.

In general, unidentified signals can be potentially identified using software-supported logical deduction based on a reasonable set of possible ingredients or by utilizing a HaVoc system based on a triple quad or a high-res mass spectrometer.

Conclusion

This study investigated the HaVoc system as a technological solution for data-driven flavor analysis. Based on the conducted measurements, we were able to confirm the stated hypotheses that the system can (a) distinguish and cluster different cherry-type flavorings from one producer and (b) differentiate between producers based on one supposing equally categorized "cherry" flavoring (H1). Furthermore, the HaVoc system demonstrated the ability to mimic and validate the results obtained by the odor perception of a human sensory panel (H2). Additionally, we showed that the system can identify the chemical compositions of cherry-type flavorings (H3).

These results indicate that the HaVoc system could be a valuable tool for the food and cosmetics industry. It not only enables quality assurance of flavorings and flavored products in terms of ingredients and contaminations, but also provides support to and relieves traditional human sensory panels in their day-to-day work. The results presented in this study indicate that the HaVoc system may even surpass the limitations of traditional panels in terms of reliability and standardized, data-driven comparability of decision results.

References

[1] Regulation (EC) No 1334/2008 of 16 December 2008

[2] EFSA, Eurobarometer on Food & Safety 2022

[3] M. Weber et al., J. Am. Soc. Mass Spectrom. 2023, 10.1021/jasms.2c00279.

©2023 Plasmion GmbH

All rights reserved

Am Mittleren Moos 48
D-86167 Augsburg
www.plasmion.de

T: +49 (0) 821 20713355
E: info@plasmion.de