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Application Note

No. 90A

Chemical

Analysis of Cigarette Odor Compounds by GC-MS, NDI, and Sensory Evaluation

Yuki Nakagawa[1], Wataru Sato[1]

Chemical

■ Abstract

A gas chromatograph mass spectrometer GCMS-TQ™8040 NX, a bench-top X-ray CT system Xseeker™ 8000, and sensory evaluation were used to analyze the odor of commercially available paper cigarettes (Fig. 1, 2). Sensory evaluation is time-consuming, expensive, and subjective, and results are influenced by environment and conditions. Therefore, an objective evaluation method must be established. The degree of filling of the shredded leaves of cigarettes is closely related to the smell, and the integrated analysis will improve the quality of cigarettes and develop new manufacturing methods.

1. Introduction

There are various kinds of cigarette leaves, such as Virginia known for its fruity note, Burley with a touch of nuts and cocoa aroma, and Oriental with its characteristic spicy smell. It is known that the aroma of the same species varies depending on the method and time of drying after harvest, temperature and humidity during fermentation, etc. Also, the composition and chemical characteristics of cigarette leaves vary from region to region, and the nutritional value and flavor of cigarette leaves can vary depending on soil composition and climatic conditions.

In addition to the natural aroma of the leaves, some brands of cigarettes have added flavoring agents to enhance the taste and aroma and to reduce the foul odor compounds that are characteristic of some cigarette leaves. There are also cigarettes with the sweet scent microcapsules in the filter.

These odors, such as leaves, artificial fragrances, and microcapsules, undergo chemical reactions when heated at high temperatures, resulting in changes in odor compounds. The combustion temperature of cigarettes is estimated to be around 800 ˚C, which causes oxidation, pyrolysis, and distillation of odor compounds. The main odor compounds of cigarettes after ignition are volatile organic compounds such as lower fatty acids, hydrogen cyanide, phenol, and naphthalene. The composition of these compounds forms the taste and odor of cigarettes. It is also known that the smell changes with time after ignition. After the flat smell after ignition has become slightly sweet with time, and the smell of oxidized smoke becomes solid and bitter in the last few minutes. Also, the clogging of the chopped leaves of cigarettes is related to smell. If the chopped leaves are too clogged, the smell of the cigarette may become strong, and it may smell pungent. On the other hand, if the shredded leaves are loosely packed, the smell of the cigarette may become faint, and the smell may not be felt very much.

Fig. 1 GCMS-TQ™8040 NX (left) and cigarette leaf sample (right)

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In this application, the odors of the leaves of three different brands of cigarettes were analyzed by the gas chromatograph mass spectrometer GCMS-TQ8040 NX using solid-phase microextraction (SPME), and the characteristic odor compounds of each brand were identified by principal compound analysis using the statistical software Multi-omics Analysis Package. The time course of odor after ignition of each brand was also measured and visualized with a plot, and correlation analysis and volcano plot analysis were performed. In addition, to confirm the relationship between the degree of filling of chopped leaves and smell, the degree of filling of chopped leaves was measured by XSeeker 8000, and integrated analysis was performed with the GC-MS measurement data.

This application note reports on the results of the principal compound analysis of three different brands, odor compound analysis including the filling degree measurement result by X-ray CT system, compound analysis including sensory evaluation of three different brands, and time course analysis of odor compounds.

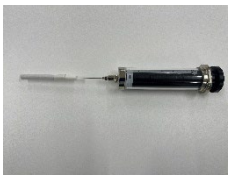
Fig. 2 Xseeker™ 8000

2. Experiments

Three different brands of commercially available cigarettes were purchased. The roll papers were unrolled, and the dried leaves were weighed into solid-phase microextraction vials at ca. 500 mg (3 cigarettes of each brand, n=3). A 50 mL gas-tight syringe was inserted into a cigarette from the filter side, 30 mL was collected every 1 minute after ignition, and 10 mL was sealed in a vial for solid-phase microextraction, as shown in Fig. 3 below.

The Smart Aroma Database™ and the Off-flavor Database can be analyzed with the same column using the same instrument setup, containing retention indicators, optimized MRM transitions, and odor characteristics (e.g., sweet, nutty, etc.).

1) Ignition



The gas-tight syringe was inserted to a depth of 2 cm from the filter side. A pen was used to mark the 2 cm point on the needle of the gas-tight syringe to collect the smoke at the same depth every time.

2) Aspiration

The plunger was pulled every minute with a time measured by a stopwatch. 30 mL was collected in about 20 to 30 seconds.

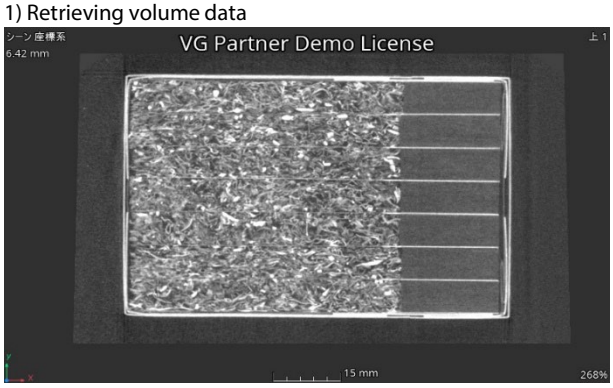
3) Putting into a tube

Of the 30 mL collected, 10 mL was enclosed in 2 separate vials; 10 mL for the Smart Aroma Database and another 10 mL for the Off-flavor Database.

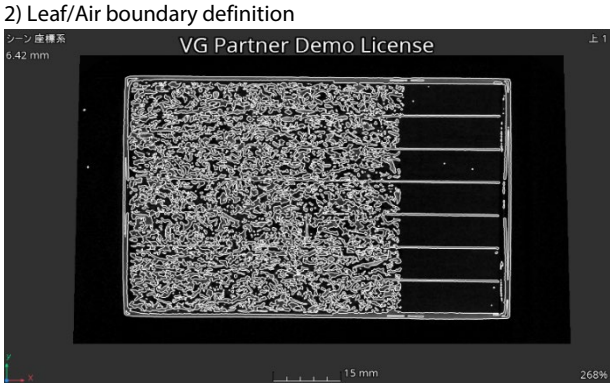
Fig. 3 Gas sampling procedure

For the fiber volume analysis using the X-ray CT system, volume data were obtained by CT imaging, and grayscale values were sorted by threshold to separate leaves (including wrapping paper) from air. (Fig. 4). The cigarette leaves were enclosed in a cylindrical shape (Φ4.6 mm×60 mm) to create a region of interest. Each region was then divided into 6 sections at an interval of 10 mm. The volume % of each region (Φ4.6×10 mm) was calculated by counting the number of "leaf " pixels in each area.

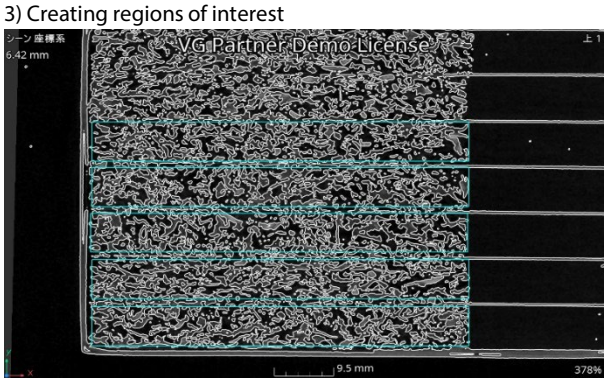
1) Retrieving volume data



2) Leaf/Air boundary definition



3) Creating regions of interest



4) Fiber volume analysis

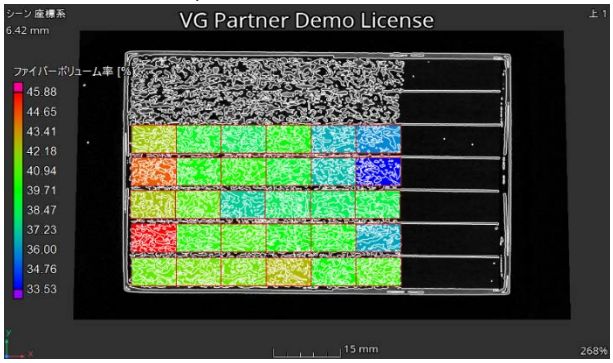


Fig. 4 Fiber volume analysis procedure

3. Characteristic compound analysis of each brand by cigarette leaves

After integrating and normalizing the 206 compounds detected by the Smart Aroma Database and the 89 compounds detected by the Off-favor Database, principal compound analysis was performed using Multi-omics Analysis Package (Fig. 5).

As shown in the loading plot in Fig. 5, many sweet compounds such as 5-methylfurfural and geraniol were detected on the right side of PC1. Therefore, the sample group shown in green was designated as "cigarette brand A (sweet cigarette)." Similarly, on the left side of PC1, 2-methylpyrazine, a nutty odor, and p-vinylguaiacol, a curry odor, were detected in the upper part of PC2, and the sample group shown in red was designated as "cigarette brand B (spicy cigarette)." The sample group shown in blue was labeled "cigarette Brand C (Standard cigarette)" because of the large amount of m-xylene, which has a plastic odor.

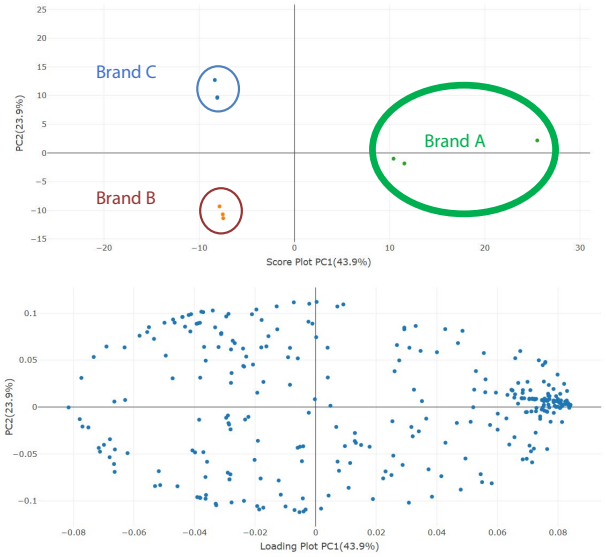


Fig. 5 Principal compound analysis of cigarette leaves

The cluster separation visually recognized with the principal compound analysis was confirmed by the dendrogram of the hierarchical clustering analysis (Fig. 6). Fig. 7 on the right shows an example of the compounds characteristic of each brand detected by the heat map. Furfural is an ingredient produced from sugar and known for its sweet aroma and was detected in high concentrations in cigarette brand A. 4-Ethyl-2 methoxyphenol, which has a spice-like aroma, was detected in high concentrations in cigarette brand B.

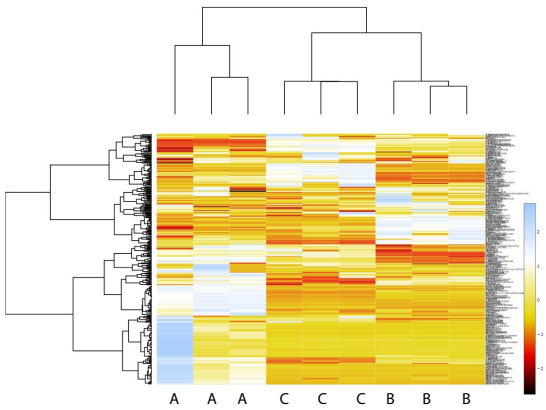
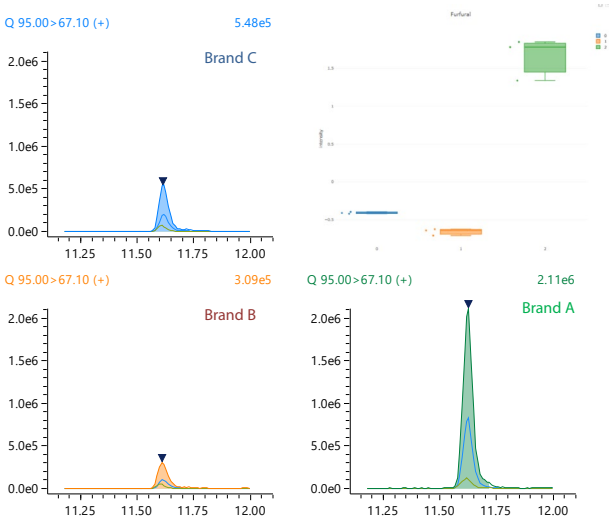


Fig. 6 Cigarette leaf class cluster analysis

sweet scent Furfural



spice-like odor 4-Ethyl-2-methoxyphenol

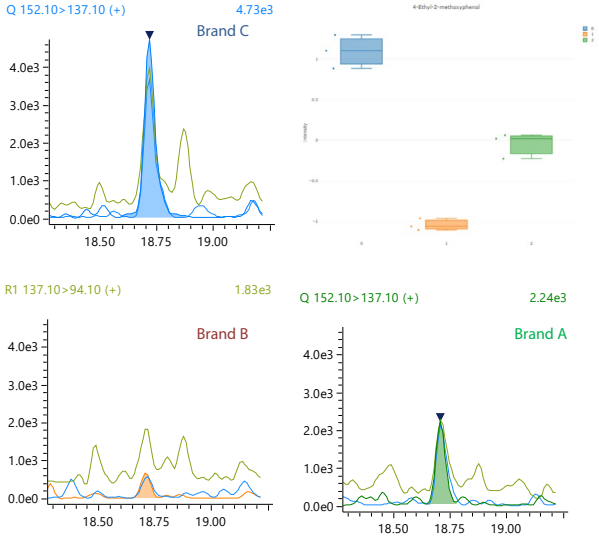


Fig. 7 Examples of odor compounds characteristic of each brand

4. Volcano plot analysis to capture changes over time

The concentrations of compounds detected within two minutes after the ignition of a sweet cigarette was compared with those of compounds detected in the last two minutes of smoking (Fig. 8). Compounds that were characteristically detected in high concentrations in the first 2 minutes are shown in green in the upper left of the plot, and compounds that were specifically detected in the last 2 minutes are shown in red in the upper right of the plot.

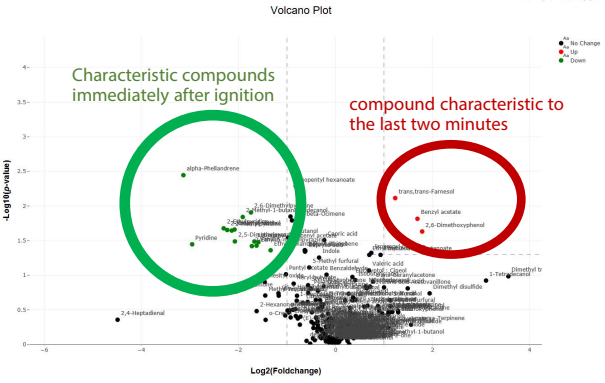


Fig. 8 Volcano plot analysis of the two minutes immediately after ignition and the last two minutes

5. Integrated analysis of X-ray CT system and GC-MS

The fiber volume of the chopped leaves obtained by XSeeker 8000 was visualized as shown in Fig. 9. We tentatively named cigarettes 1, 2, 3, 4, and 5 from the bottom. Since the cigarettes 2 and 4 had similar filling distributions as did 3 and 5, we measured the cigarettes 1, 2, and 3 by GC-MS using Smart Aroma Database, and Off-flavor Database.

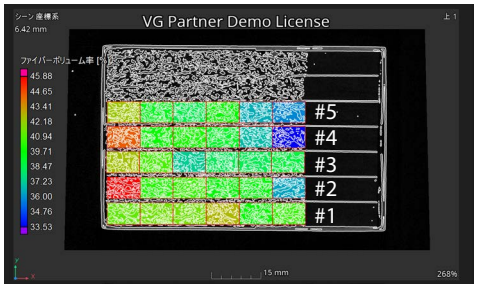


Fig. 9 Sample names and locations of cigarettes

The cigarettes 1, 2, and 3 were ignited, and smoke was collected from the tip with a gastight syringe at an equal interval from the burning tip. The portion that XSeeker 8000 identified as having a high degree of filling (cigarette 1 41-50 mm, cigarette 2 1-10 mm, cigarette 3 1-10 mm) was compared with the remaining portion in 2 groups using a volcano plot. In the upper left of the plot (green box), compounds are more abundant in the low fiber-volume region (Fig. 10).

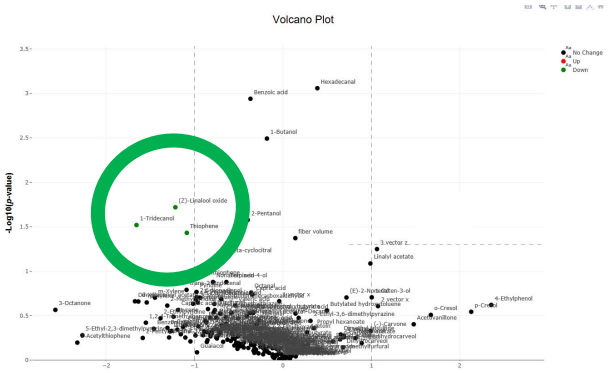


Fig. 10 Comparison of high- and low-filling portions of cigarette 1 to 3

In addition, PCA was performed to evaluate whether the tip 1-10 mm portion, which has a high fiber volume, is statistically different from the other portions of the cigarette 2 (Fig. 11). A good result was obtained with a cumulative contribution of about 60 %. When the high and low filling areas were visualized in box plots for each compound, it was found that a high concentration of burnt odor compounds (e.g., 2-Cyclohexen-1 one) was detected in the high fiber volume region (Fig. 12).

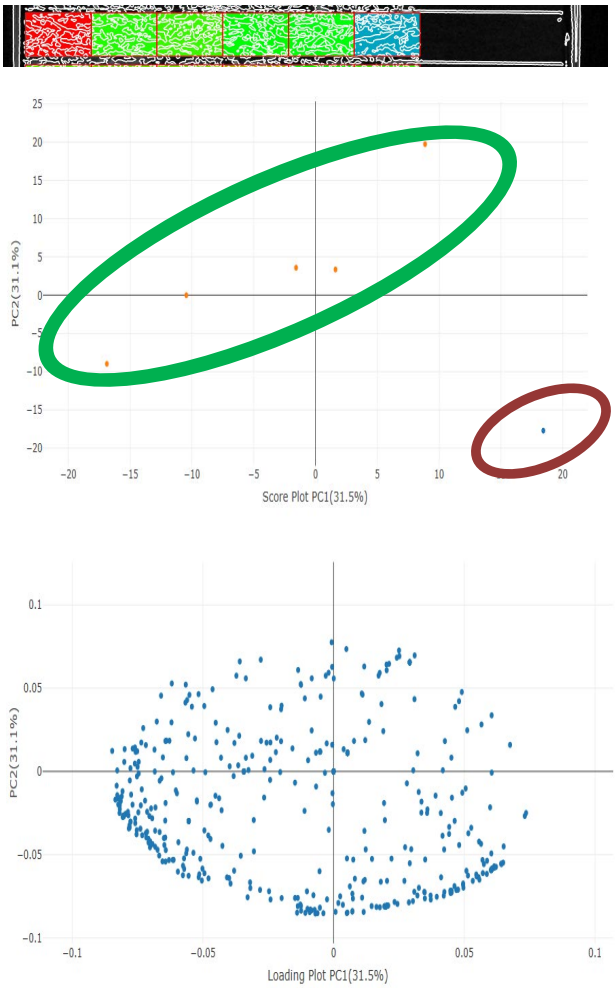


Fig. 11 Comparison of the high and low fiber volume regions of the cigarette 2

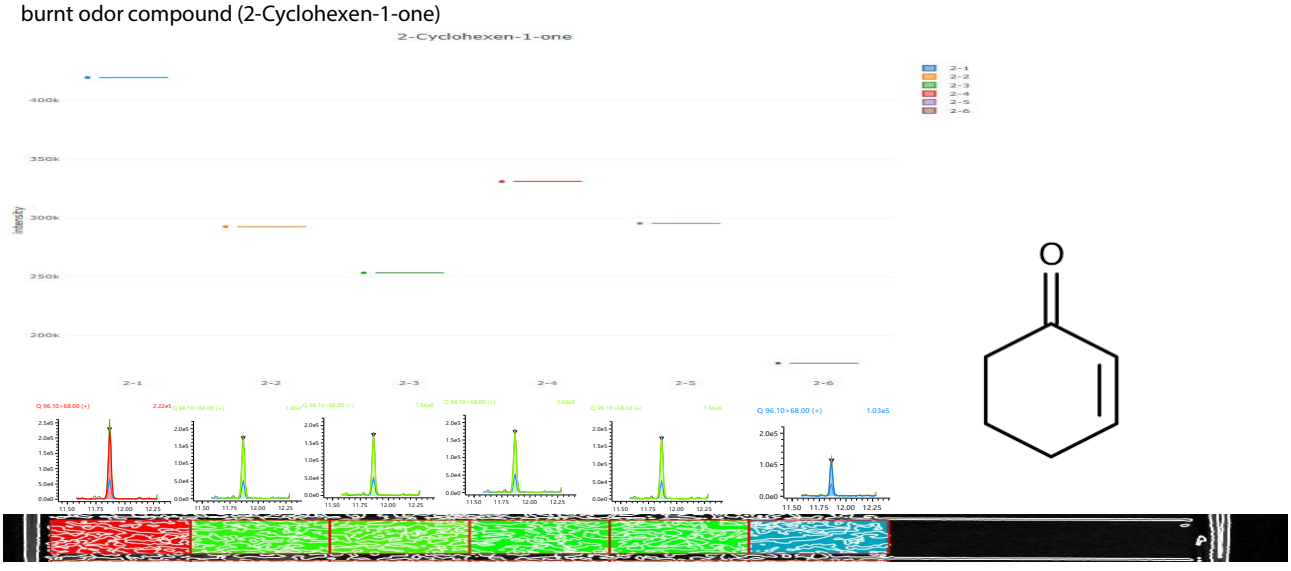


Fig. 12 Relationship between the degree of fiber volume of the cigarette 2 and the strength of odor compound after ignition (burnt odor compound 2-Cyclohexen-1-one)

In addition, when the cigarette 3 was analyzed in the same way, hexadecanal, an odorless compound, was detected at a high fiber volume region in the tip part 1-10 mm. Hexadecanal is known as an odorless chemical that changes mood when people sniff.

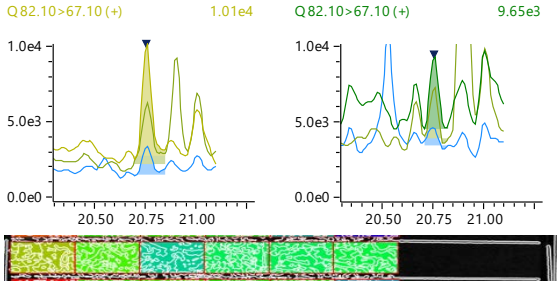


Fig. 13 Relationship between the degree of fiber volume and odor compounds of the cigarette 3

However, comparing each cigarette with each odor compound in this way is a complicated task. As shown in Fig. 14 below, the vertical axis is the odor compounds measured by GC-MS, and the horizontal axis is the degree of fiber volume measured by the X-ray CT system. By drawing a heat map of each cigarette, we integrally analyzed the degree of fiber volume and detected about 300 odor compounds simultaneously. The analysis in the figure below uses the cigarette 2 as the horizontal axis. Many odor compounds were detected at high concentrations in the tip part 1-10 mm area with a high fiber volume. On the other hand, these compounds are estimated to be highly correlated with the fiber volume because they are detected at low levels in the 51-60 mm region farthest away from the tip with low filling. It was also found that some compounds were highly detected only in the 11-50 mm area, where the degree of filling was moderate. Further analysis of these compounds, including bitter, sweet, and astringent compounds, will lead to improvements in the quality of cigarettes and the development of new manufacturing methods.

In the heat map, each cigarette was analyzed one by one, but by using the mapping feature of Multi-omics Analysis Package, multiple cigarettes can be plotted as a bar graph at the same time, as shown in Fig. 15 below. The correlation analysis function can automatically color-code (Red for high positive correlation, blue for high negative correlation, light for no correlation) the correlation with odor compounds in a few seconds.

The red color is the cigarette 1, the green color is the cigarette 2, and the blue color is the cigarette 3. The fiber volume value (filling degree) was imported into the map. Because the degree of dispersion between the degree of filling and the area value of GC-MS differs in absolute value, the degree of filling and the area value of each compound were normalized before being fed into the map. It was found that acetovanillone had a strong positive correlation, while ethyl stearate had a strong negative correlation.

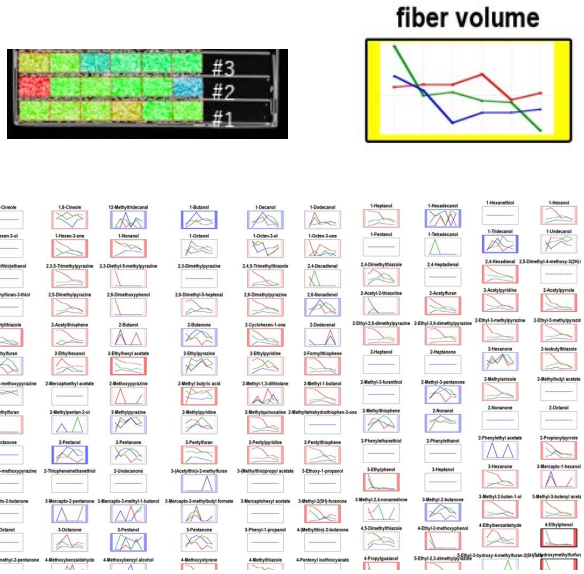


Fig. 15 Relationship between the fiber volume and odor compounds of the cigarettes 1 to 3

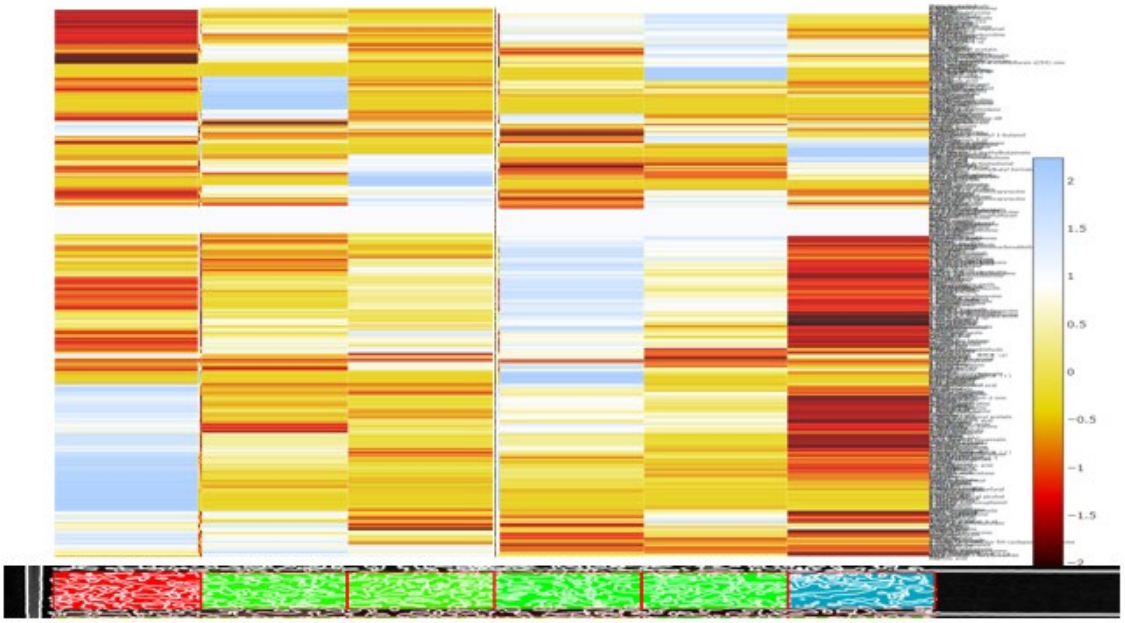


Fig. 14 Relationship between the degree of filling and odor compounds of the cigarette 2

6. Sensory evaluation and correlation analysis of each compound

The sensory evaluation of the leaves of each cigarette brand was conducted by three people of different ages and genders on four features: strength, sweetness, bitterness, and sourness. Each feature was rated on a 3-point scale (i.e., none, somewhat, strong), and the average values of the 3 subjects were tabulated (Table 1). In the sensory evaluation, even a spicy cigarette has a sweet taste and is rated as high as 2.7. Standard cigarettes have the lowest overall odor intensity, rated at 2.3, and the lowest sweetness at 2.3. Sweet cigarettes have a high sweetness of 3.0 but a low bitterness of 1.0.

In Multi-omics Analysis Package, the results of each compound's relative quantitative values and each brand's sensory evaluation were visualized as bar graphs (Fig. 16). The red bar graph is for the spicy cigarette, the blue bar graph is for the standard cigarette, and the green bar graph is for the sweet cigarette. The visualized data were analyzed using the relative analysis function in Multi-omics Analysis Package, which automatically colors the highly correlated compounds with the sensory evaluation.

In Fig. 16, odor compounds with high correlation were automatically color-coded using "sour taste" as the standard sensory evaluation. It has been determined that the red background color compound of the bar graph is highly positively correlated, and the blue background color compound is highly negatively correlated.

Table 1 Sensory Evaluation of Cigarette Leaves (Mean of 3 individuals)

	Strength	Sweetness	Bitterness	Sourness
Spicy brand B	2.7	2.7	1.7	1.3
Standard brand C	2.3	2.3	2.0	2.0
Sweet brand A	3.0	3.0	1.0	1.3

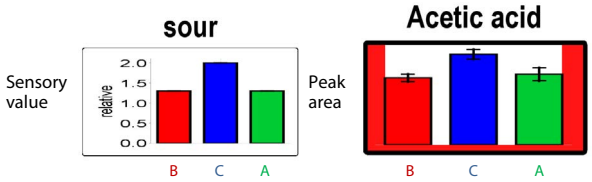


Fig. 16 Sensory evaluation "sourness" and odor compound acetic acid

Correlation coloring is gradient-colored by correlation strength, with pink indicating a weak positive correlation and light blue indicating a weak negative correlation.

A strong positive correlation was found for acetic acid based on sour taste (Fig. 17). A scatter plot showing the correlation between acidity and acetic acid showed a strong correlation of R=0.92 (Fig. 18).

In the same way, we conducted a correlation analysis on "strength," "sweetness," and "bitterness" and found a strong correlation with 5-methyl furfural, cyclotene, and 2,3-trimethylpyrazine, respectively.

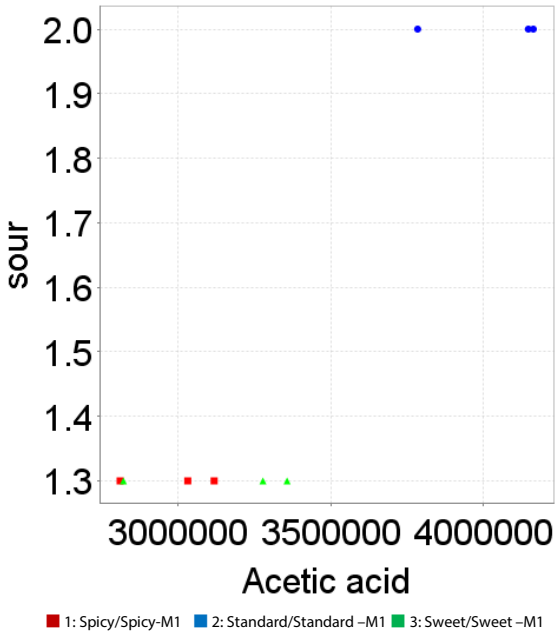


Fig. 18 Correlation between sensory evaluation "sourness" and odor compound acetic acid

Fig. 17 Sensory evaluation of cigarette leaves (mean of 3) and visualization of relative quantification of each odor compound

7. Puff-by-puff analysis of a lit cigarette

The gas sample measurement data was also visualized using Multi-omics Analysis Package as in the previous section. The horizontal axis indicates each puff after ignition, and the vertical axis indicates the area value of the compound. The red line graph is for the spicy cigarette, the blue for the standard cigarette, and the green for the sweet cigarette. The software's correlation analysis function automatically color-coded compounds showing an upward trend with a red background color and compounds showing a downward trend with a blue background color (Fig. 19).

The concentration of dimethyl trisulfide, an organosulfur compound, increased with each inhalation (Fig. 20). Dimethyl trisulfide is known as a malodorous ingredient, such as rotten cabbage, and it is assumed that the concentration of the substance per breath increases over time after ignition, which is unfavorable to consumers. Similarly, valeric acid, a pungent odor compound such as cheese odor and sweat odor, and thiophene, an unpleasant odor such as gasoline, were detected.

The compounds that decreased after ignition included ethyl hexanoate, an apple odor, and 1-butanol, a sweet fruit odor. It was found that the sweet odor compound decreased over time.

8. Conclusion

This study evaluated three different shredded cigarette leaves and ignited gas samples from multiple angles using a gas chromatograph mass spectrometer (GCMS-TQ8040 NX), a bench-top X-ray CT system XSeeker 8000, and sensory evaluation. Using a Multi-omics Analysis Package, we were able to detect the characteristic odor compounds of each brand and filling degree by integrated analysis of the fiber volume measured by the X-ray CT system, 295 compounds detected by GC-MS, and sensory evaluation values using principal compound analysis, hierarchical clustering analysis, volcano plot analysis, and correlation analysis. We conducted four analyses: a principal compound analysis of three different brands, an odor compound analysis by the filling degree measured with the X-ray CT system, a compound analysis using sensory evaluation of three different brands, and a puff-by-puff analysis of odor compounds. These four analyses enabled a comprehensive analysis of cigarette product.

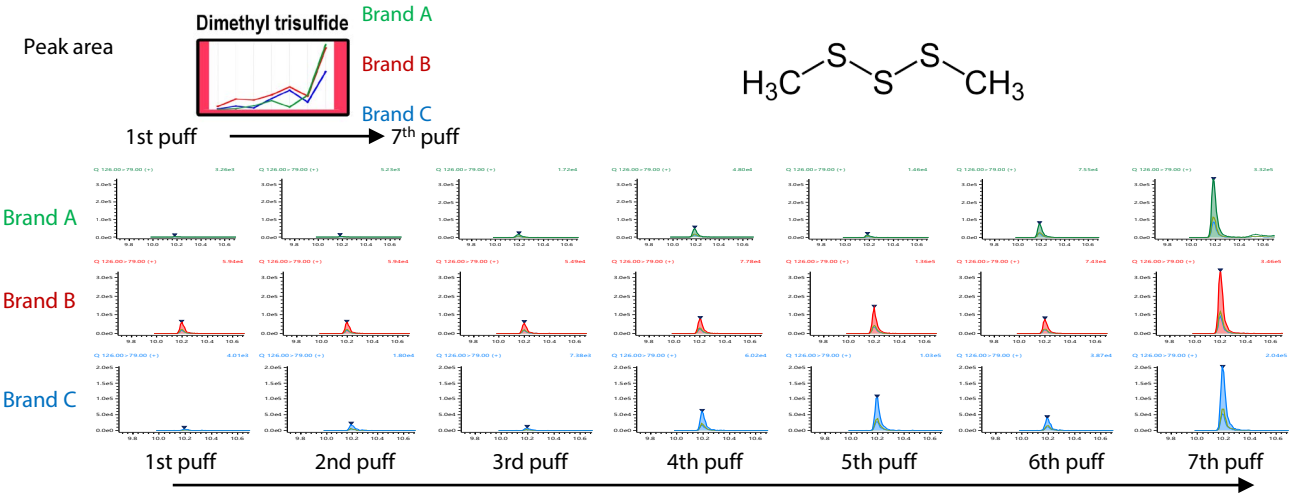


Fig. 19 Change in the concentration of dimethyl trisulfide after cigarette ignition for each puff inhalation

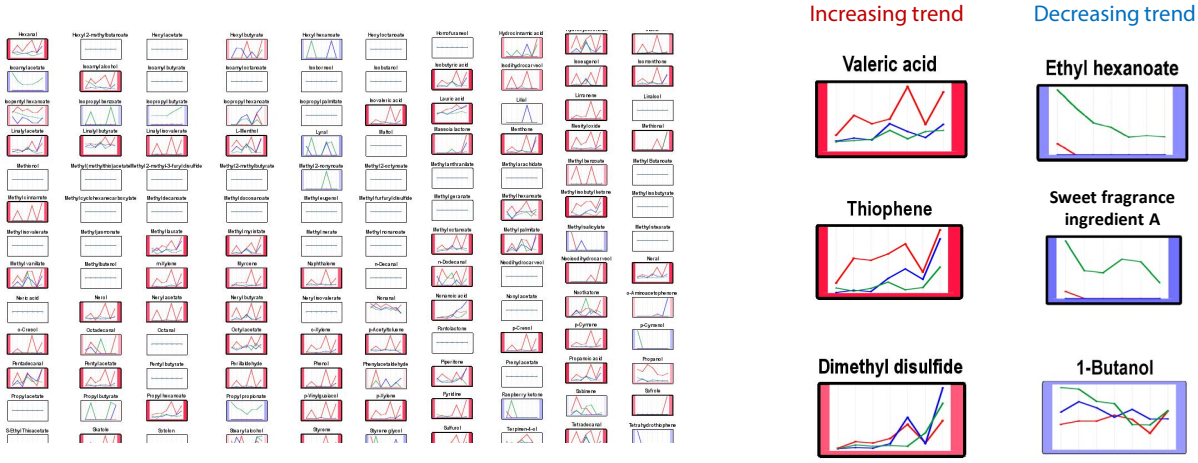


Fig. 20 Change in concentration for each puff inhalation from cigarette ignition

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