Original Article

A Short-Term Time-Series Data Analysis Algorithm for Flavor Release during the Start of Eating

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"Retronasal aroma" refers to the aroma released from food during consumption and traveling through the nose after leaving the mouth. It is closely related to the behavior of odor compounds released from food into the mouth and plays a crucial role in our overall perception of flavor. As a result, research focusing on measuring the behavior of retronasal aroma has gained attention for exploring the relationship between sensory perception and flavor. We attempted to develop a data analysis method that specifically targets a time span of a few seconds to tens of seconds, starting from when food is placed in the mouth during eating and extending to just after swallowing. In this study, we observed a strong correlation between the periodic waveform data derived from performing the third derivative (jerk) on the detection intensity data obtained using a mass spectrometer and the behavior of the detection intensity. Furthermore, by performing a frequency analysis using a fast Fourier transform on the jerk data, it was possible to extract the frequencies that contribute to sensory perception during eating. Furthermore, the reconstructed jerk data derived from the extracted data using the inverse fast Fourier transform provided a clearer explanation of sensory perception during eating. Our algorithm suggests new short-term time-series data applications.

INTRODUCTION

The aroma of food is an important factor that affects its flavor composition. Exploration of aroma compounds that contribute to flavor has been actively pursued using gas chromatography–mass spectrometry (GC/MS). In recent years, growing attention has been to retronasal aroma, which refers to the aroma released from food into the nasal cavity during eating. Understanding the retronasal aroma, which is influenced by the release of aroma compounds from food in the mouth and is affected by the eating process, plays a crucial role in studying food flavors. Therefore, to study retronasal aroma, it is necessary to analyze the flavor-release behavior of aroma compounds from food in real time during the release process.

However, conventional GC/MS is not capable of real-time analysis of time-series data. Therefore, real-time measurement systems for flavor release using an atmospheric pressure chemical ionization (APCI) ion source have been studied, as reported by Linforth *et al.*,[1] and have led to various proposals for flavor-release measurement systems and analytical methods.[2] Currently, the proposed measurement systems are based on mass spectrometry (MS) techniques and often combine dedicated sampling devices with APCI-like ion sources, with proton transfer reaction mass spectrometry as a representative example. Our group has also proposed a highly versatile measurement system that combines a direct analysis in real time (DART) ion source[3] with liquid chromatography mass spectrometry (LC–MS).[4]

When considering data analysis methods, it is necessary to establish a connection between measurement results and the human perception of odor as well as determine the appropriate approach for handling time-series data.

Based on this perspective, analysis methods focusing on the postswallowing state during eating are widely used. This period aligns with the timeframe of the aftertaste, which plays a significant role in the overall flavor recognition process. Specifically, the method involves calculating the amount of aroma components released from the nostrils after each breath following swallowing while eating as the area under

the curve (AUC) value and analyzing them in a chronological order.[5,6] Based on this idea, data processing methods have been proposed to relate measurement data to flavor perception.[7,8] In addition, during the flavor recognition process while eating, the flavor is perceived even during the short period between placing the food in the mouth and swallowing. Therefore, a method has been proposed to relate sensory evaluation results using the detection curve of aromatic compounds from the start of eating to the aftertaste, focusing on the entire eating process and using the maximum intensity, time, and overall AUC values.[9] Recently, this approach has been applied not only in food research but also in studies focusing on the decline of olfactory perception in the elderly from an oral physiological perspective.[10,11]

Despite various studies on measurement and analysis methods, research on data analysis methods specifically targeting the period between chewing and swallowing remains relatively underdeveloped. This gap can be attributed to the challenge of analyzing the behavior of retronasal aroma, which occurs on a sub-second timescale and requires real-time continuous measurement, given that chewing and swallowing occur within a brief period of a few seconds.

Current analysis methods have a time resolution on the order of a few seconds, significantly higher than the sub-second resolution required to capture a single breath. Furthermore, the reproducibility of eating on a second-by-second basis during repeated measurements is difficult to achieve, and even slight variations in movement that do not significantly affect flavor perception can have a large impact on the measurement results.[12] To address this uncertainty, research on simulators[11,13] that aim to reproduce chewing and swallowing movements has also been conducted. However, at present, no simulator exists that can reproduce all eating movements continuously in a short time period. Consequently, despite the fact that flavor recognition occurs during the eating process in this time period, research in this area has not progressed because of the recognition of this unstable measurement data region.

Therefore, we aimed to develop a data analysis method that targets extremely short time-series data, ranging from a few seconds to tens of seconds, from placing food in the mouth to immediately after swallowing. To relate the detection intensity obtained by MS to sensory intensity in flavor recognition, we adopted the idea of the "minimum jerk model" proposed by Flash and Hogan.[14] This idea converts the smoothness of motion in the movement domain into a semi-quantitative value and correlates it with a sensory quantity. We attempted to correlate the third derivative value of the detection intensity history obtained by measuring the sensory intensity of the smell. As a result, we confirmed that it did not contradict the Weber–Fechner's law, which states that "the amount of stimulation that humans perceive as different is proportional to the logarithm of the stimulus intensity." In order to overcome the challenge of synchronizing measurements at 1-s intervals during the eating process, we attempted to convert it into the frequency domain, which is not affected by the time axis. The jerk data in this study refer to waveform data with periodicity centered around zero, obtained by converting the detection intensity data from a mass spectrometer. A fast Fourier transform (FFT) was then performed, transforming the time-series data into frequency-domain data
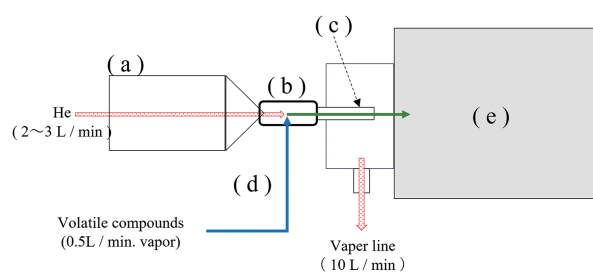

Fig. 1. Analytical system for flavor release using DART ion sauce and Volatimeship. (a) DART ion sauce, (b) Volatimeship for ionization, (c) ceramic tube, (d) installation line of volatile compounds, and (e) mass spectrometer LC–MS 8040. DART, direct analysis in real time; LC, liquid chromatography; MS, mass spectrometry.

with its corresponding power spectrum, which was independent of the time axis. As a result, it was confirmed that semi-quantitative comparative analysis is possible even for measurement data that are not synchronized at a 1-s interval. Furthermore, by utilizing orthogonal partial least squares discriminant analysis (OPLS-DA), it was possible to separate the frequencies that contributed to the sensory intensity from those that created noise in the frequency data. By reconstructing these data into jerk data using an inverse fast Fourier transform (IFFT), it was also confirmed that noise resulting from slight variations in the subjects' chewing movements can be removed. Therefore, the developed preprocessing algorithm is suggested to be effective for retronasal aroma measurement, and its development is discussed in this work.

EXPERIMENTAL PROCEDURES

Instruments
DART–MS analysis system

A DART ion source (IonSense, Saugus, MA, USA) was employed coupled to an LC–MS 8040 (Shimadzu, Kyoto, Japan), a triple quadrupole mass spectrometer. A Volatimeship (BioChromato, Fujisawa, Japan) was used as the sample introduction system between the DART source and the mass spectrometer (Fig. 1). Note that the Volatimeship was modified to allow the intake of outside air into the ionization chamber to enable the measurement of the retronasal aroma (Fig. S1). The analytical conditions were as follows: DART ion source temperature: 300°C, helium (99.99% purity): 10 L/min, and LC–MS 8040 was operated in multiple reaction monitoring modes (l-carvone: 151>109, collision energy: −20 kV, dwell time: 20 ms).

Flavored beverage samples

For DART–MS analysis, two low-viscosity gel aqueous solutions with different rheological characteristics were prepared to create flavored beverage samples. Xanthan gum and tamarind gum (DSP Gokyo Food & Chemical, Osaka, Japan) were used as gelling agents. The flavored components were l-carvone and d-limonene (food grade), and the blend was formulated to have the aroma characteristics of spearmint oil. The reference spearmint oil used in this study was subjected to GC–MS analysis to confirm the ratio of l-carvone and d-limonene used. The gel made with xanthan gum was named xanthan gum-based flavored gel (XFG), and the gel made with tamarind gum was named tamarind gum-based
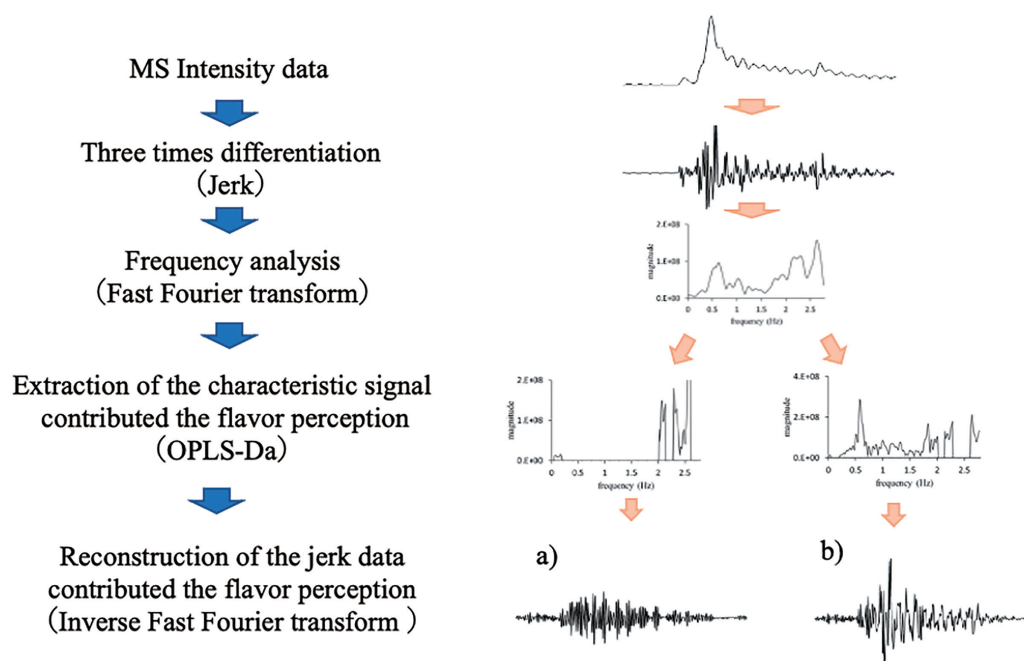
Fig. 2. Algorithm to extract and reconstruct characteristic signals. (a) Jerk signal with a large sensory contribution. (b) Jerk signal with little contribution to the sensory. MS, mass spectrometry; OPLS-DA, orthogonal partial least square-discriminant analysis.

flavored gel (TFG). The composition of the two blends was as follows:

- XFG: xanthan gum (0.3%), *l*-carvone (0.012%), *d*-limonene (0.004%), granulated sugar (5%), and water (94.684%).
- TFG: tamarind gum (0.5%), *l*-carvone (0.012%), *d*-limonene (0.004%), granulated sugar (5%), and water (94.484%).

The granulated sugars were used to disperse the gelling agents. To avoid the volatile loss of the flavored components from the gel during adjustment, *l*-carvone and *d*-limonene were added after cooling the gel to room temperature, and the mixture was stirred to homogenize. The amounts and balances of the flavored components in the two gels were confirmed to be the same (Fig. S2a and S2b). XFG was a non-Newtonian fluid with high viscosity at rest, whereas TFG was a Newtonian fluid with low viscosity at rest. When the adjusted gels were stirred at a high shear rate, their viscosities remained almost constant (Fig. S2c). A sensory evaluation of the two flavored beverage samples was conducted using a panel of five individuals. All panelists confirmed that the TFG had a stronger cooling sensation than the XFG (data not shown).[15,16]

Retronasal aroma analysis

A gel solution was ingested orally, and the volatile components that were released from the oral cavity into the nostrils during the breathing process, with air introduced into the mouth at a rate of 2 s per cycle, were continuously measured in real time. The sampling method involved placing the end of the transfer line connected to the ion source against the nostrils and inhaling the volatile components released from the nostrils. The connection between the nostrils and the transfer line was not sealed, and a slight gap was left to allow for the inflow of ambient air into the nasal cavity and transfer line. The gel sample was ingested as follows: 1) a flavored beverage sample was introduced into the oral cavity

and allowed to remain for 3 s (feed-on status was defined); 2) the sample was then swallowed rapidly in approximately 2 s (swallowing status was defined); 3) after swallowing, breathing was performed while keeping the mouth open to allow air to enter the oral cavity (last note status was defined); and 4) the measurement was completed 60 s after introducing the flavored beverage sample into the oral cavity. Before introducing the gel into the oral cavity, the subjects breathed while keeping their mouths open to allow the introduction of air (blank status was defined). Measurements were performed by one subject, and each measurement was performed with a gap of at least 5 min to avoid the influence of other volatile components remaining in the oral cavity. After each measurement, the participants rinsed their oral cavities with mineral water and unscented carbonated water. Unscented carbonated water was used because it has a high cleaning power for aromatic components in the oral cavity when used in combination with mineral water. Before proceeding to the next measurement, a blank measurement was performed to check if any target aromatic components remained in the oral cavity without anything in the mouth. If any target aromatic component was detected, another blank measurement was performed after waiting for an additional 5 min.

Processing of measurement data

Latent signal extraction using jerk and Fourier transform

We performed an analysis using data obtained by DART–MS and measured *l*-carvone released from the nasal cavity during the tasting of XFG and TFG, collecting six replicates for each tasting. The data processing steps (Fig. 2) were as follows. 1) We performed three differentiations of the absolute ion intensity data of *l*-carvone obtained by MS to obtain the jerk values, with each differentiation calculated using two adjacent data points. 2) We used 128 data points, including the time period in which there was a difference in coolness
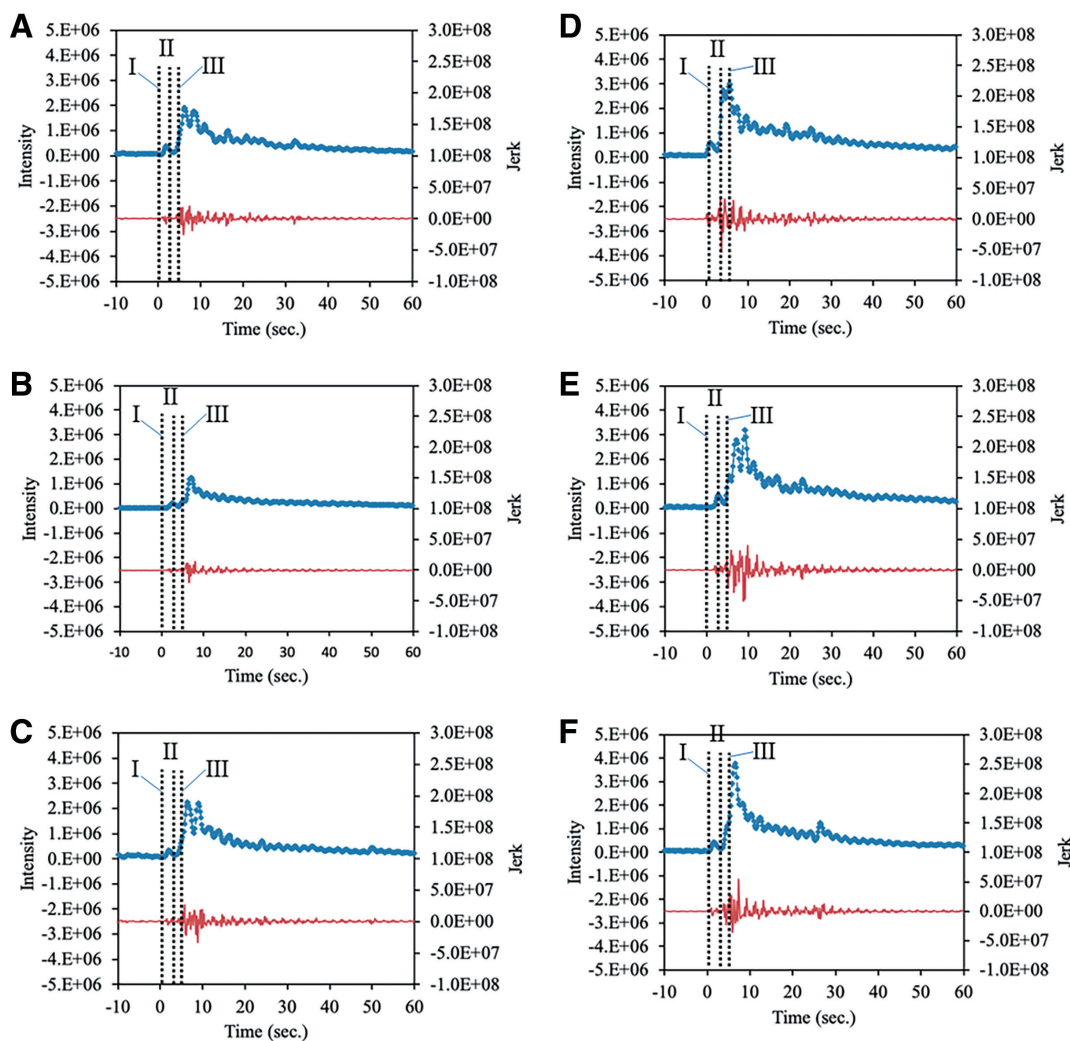
Fig. 3. Total ion current chromatogram and the jerk graph on the retronasal aroma of drinking flavored gel samples. The top is the TICC and the bottom is the jerk graph. The first axis shows the intensity, and the second axis shows the jerk. Each TICC is measured in the MRM mode (151>109). Each jerk graph was drawn based on the intensity data shown. (A), (B), and (C) are graphs of xanthan gum-based gel (xanthan gum 0.3%). (D), (E), and (F) are graphs of tamarind gum-based gel (tamarind gum 0.5%). I, II, and III indicate the process to eat. Time periods from I to II indicate feeding status. Times from II to III indicate swallowing status. Periods after III indicate the effects of swallowing. MRM, multiple reaction monitoring; TICC, total ion current chromatogram.

sensation according to the sensory evaluation (5–10 s), to perform frequency analysis using FFT with a Hamming window used as the window function.[17] 3) We then conducted an OPLS-DA using XFG and TFG as response variables and the frequency and power spectra obtained by frequency analysis as explanatory variables. After conducting the OPLS-DA, we selected the frequencies with the largest contributions to differentiate between the two samples and defined them as "sensory contribution frequencies" (Fig. S3). 4) We then expanded the data points to 256, performed an FFT in the same manner, and selected the data for the sensory contribution frequencies to reconstruct the jerk values using IFFT. We refer to this process as the "Jerk-FFT procedure." The reason for performing the Fourier transform in this process is that the target data are time-series data that change in 1-s units, and when comparing each datum using multivariate analysis, subtle time differences can occur, making it difficult to compare using multivariate analysis. Therefore, in the analysis, it is important to represent the detection intensity curve as frequencies and their strengths (power spectra), which are not related to the time axis. Additionally, for Fourier

transform-based frequency analysis, it is desirable to have the waveform data centered around 0. To achieve this, it is necessary to convert the detection intensity data into jerk data by performing three differentiations. We chose to perform three differentiations in the field of motion based on a study by Flash and Hogan.[14] Specifically, we calculated the difference in the coolness sensation during the time period from 5 to 10 s, when it was most strongly perceived. For intensity data, we calculated the AUC. The jerk data were calculated using the minimum jerk model concept based on Flash and Hogan's equation, regardless of the algorithm used.

$$C = \frac{1}{2} \int_{t_1}^{t_2} \left(\left(\frac{d^3 x}{dt^3} \right)^2 x \right) dt$$

RESULT

Three characteristic features were observed from the results obtained using the two types of flavored gels (n=6), as
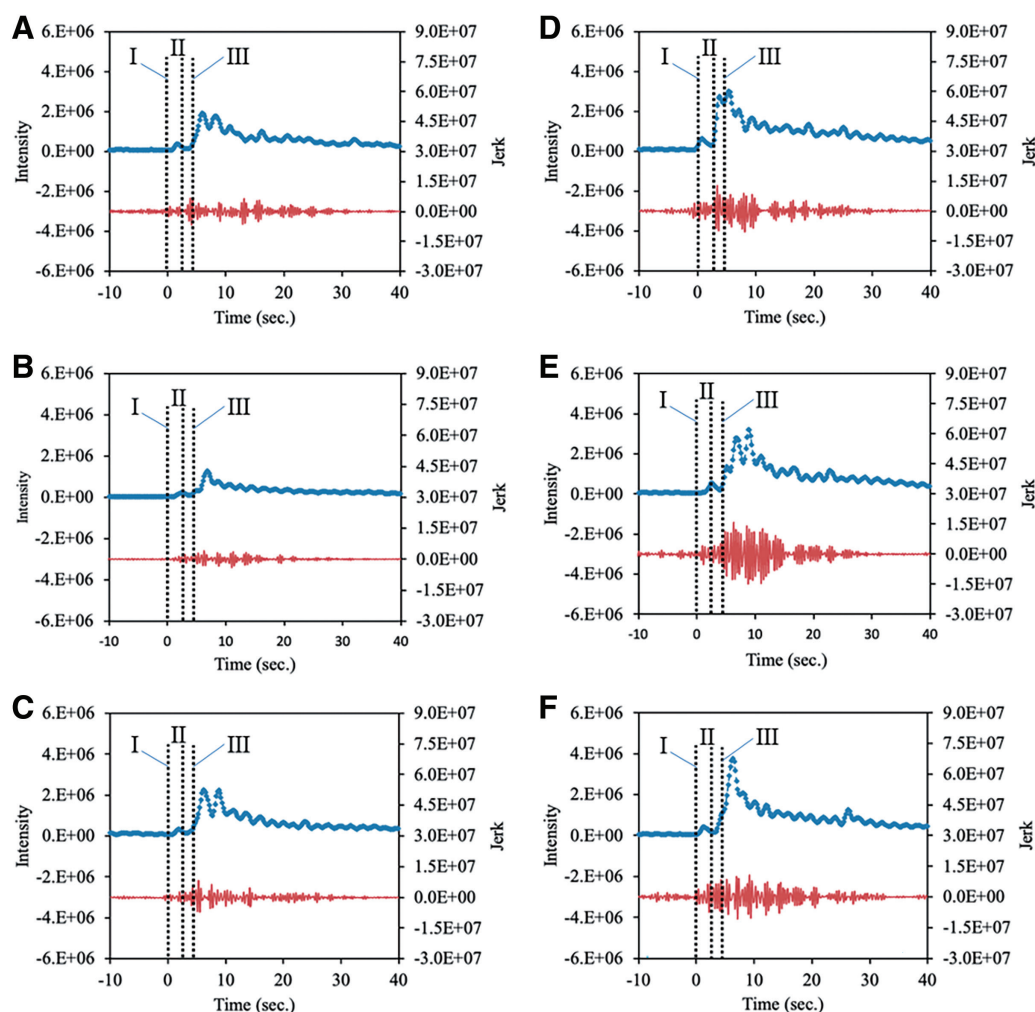
Fig. 4. Total ion current chromatogram and the jerk graph after algorithmic processing on the retronasal aroma of drinking flavored gel samples. The top is the TICC and the bottom is the jerk graph. The first axis shows the intensity, and the second axis shows the jerk. Each TICC is measured in the MRM mode (151>109). Each jerk graph was drawn based on the intensity data shown. (A), (B), and (C) are graphs of xanthan gum-based gel (xanthan gum 0.3%). (D), (E), and (F) are graphs of tamarind gum-based gel (tamarind gum 0.5%). I, II, and III indicate the feeding process. Time periods from I to II indicate the ingestion status. Times from II to III indicate the swallowing status. Periods after III indicate the effects of swallowing. MRM, multiple reaction monitoring; TICC, total ion current chromatogram.

shown in Fig. 3. The graph depicted in the upper portion of the figure shows the intensity of the detection behavior when *l*-carvone was released from the nostrils, and the lower part shows the jerk calculated based on the intensity. By comparing the intensity data, it can be confirmed that the TFG generally has a larger maximum strength. However, according to the Weber–Fechner's law, which states that psychological sensation is perceived in proportion to the common logarithm of the stimulus intensity, the difference is too small to explain the result of "clearly perceived coolness" obtained from the sensory evaluation. Therefore, based on the concept of the minimum jerk model, which considers jerk instead of the intensity behavior of *l*-carvone detection, it was confirmed that the amplitude of the jerk graph exhibits a behavior similar to that of the intensity graph. In the minimum jerk model in the motion domain, a smaller jerk indicates a smoother movement. Therefore, considering the results of the sensory evaluation, it can be said that the magnitude of the jerk shows a behavior similar to that of the sensation intensity. Furthermore, the difference in amplitude between the two flavored gels became larger compared to the intensity graph, and the result was closer to the

sensory evaluation. However, despite the fact that the sensory evaluation clearly indicated that the TFG had stronger coolness, there were cases where the difference in jerk was small, as shown in Fig. 3C (XFG) and 3F (TFG). There is some variation in eating behavior even when repeated by the same subject.[5,18] Therefore, we attempted to remove the noise caused by subtle breathing variations that did not contribute to sensation from the obtained data. Specifically, an FFT was performed on the jerk data to convert them into frequency data independent of the time axis. Discriminant analysis (OPLS-DA) was performed using the data to extract the frequencies that contributed to the sensation (Fig. S3). The extracted frequency data were then subjected to the IFFT to reconstruct the jerk (Fig. 2). Consequently, the shape of the reconstructed jerk graph is different from that before reconstruction, and the difference between the XFG and TFG is clearly shown. In addition, the behavior of *l*-carvone, which was released slightly from the nostrils after swallowing (10–40 s) and could not be confirmed in the jerk graph before reconstruction, was observed (Fig. 4). Next, we performed a semiquantitative comparison. The intensity data were compared using the AUC values, while the jerk
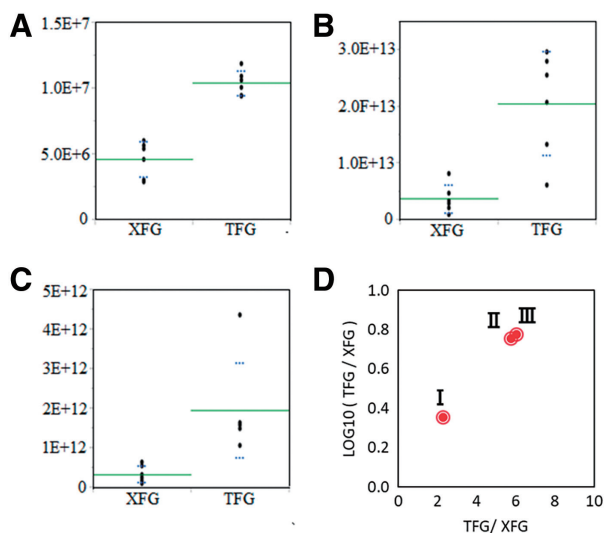
Fig. 5. Comparison of the values calculated using three types of data at drinking flavored gel samples. Graph (A) shows the area calculated from the intensity curve data, graph (B) shows the area calculated from the square of the jerk values, and graph (C) shows the area calculated from the square of the jerk values after FFT–IFFT preprocessing. The solid line shows the mean of each sample, and the dashed line shows its standard deviation. Graph (D) shows the relationship between the TFG/XFG ratio and its common logarithm calculated from graphs (A), (B), and (C). I, II, and III show the different conditions used to determine the TFG/XFG ratio, calculated, respectively, from the detection intensity, the jerk value, and the jerk value after reconstruction. FFT, fast Fourier transform; IFFT, inverse fast Fourier transform; TFG, tamarind gum-based flavored gel; XFG, xanthan gum-based flavored gel.

data before and after reconstruction were calculated based on the minimum jerk model (Fig. 5). When comparing the AUC values obtained from the intensity data, XFG and TFG were sufficiently distinguished (Fig. 5A). However, the common logarithm of TFG/XFG using the mean values of each data point was only 0.36, indicating a small and thus insignificant difference in sensation according to the Weber–Fechner's law. Using the mean value of each data point after jerk processing, the common logarithm of TFG/XFG was 0.76, indicating a large difference in sensation (Fig. 5D). However, there was an overlap in the data between the XFG and TFG, making it insufficient to distinguish between the two groups (Fig. 5B). The common logarithm of TFG/XFG obtained using the mean values of the reconstructed jerk data was 0.78 (Fig. 5D), and the XFG and TFG data were clearly distinguishable (Fig. 5C).

DISCUSSION

We aimed to visualize the aroma profile during short-term movements after consumption at 1-s intervals. It is difficult to maintain consistent movements at 1-s intervals even within the same individual, from consumption to swallowing. Therefore, reducing the variation in the detection intensity data obtained through MS is an important challenge in visualizing these behaviors. In this study, we attempted to remove sensory-unrelated noise from the results using samples that showed significant differences in the cooling sensation in the sensory evaluation. We developed a method for extracting information that may contribute to the

senses from measurement data. The jerk, which is the third derivative of the original intensity data, showed an aroma profile during consumption, and its amplitude strength and semi-quantitative values using the minimum jerk model were found to be related to sensory intensity. Furthermore, by applying frequency analysis with FFT to the jerk data, multivariate analysis approaches such as OPLS-DA became possible. Subsequently, significant sensory-contributing data were extracted, and the jerk data were reconstructed by IFFT. The reconstructed jerk data were more closely related to the sensory differences than to the original intensity data in the unprocessed analysis. The time-series curves of detection intensity in MS may be a collection of complex data influenced by various factors, such as respiratory and oral movements during consumption. This was suggested by the results of frequency analysis using FFT, which can extract and reconstruct data with high regularity at specific frequencies. Although this experiment was conducted on specific subjects using a single-model system, further verification is necessary. However, using the Jerk-FFT procedure on the same subjects enabled the reduction of variation in consumption movements and the extraction of information significantly related to the senses, which is a significant contribution. The concept proposed in this study suggests the potential for new developments in the analysis of short-term time-series data, while also considering its potential to evolve into an effective tool for product design in the food industry, allowing the control of flavor perception.

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