Suppression of Interference of Fixative With Odorant Samples in Mass Spectrum Using ICA

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ABSTRACT Odor sensing enables us to sense odorant stimuli. This sensation causes a deep subconscious response in humans in various ways and represents an indispensable sensation in daily life. However, unwanted compounds called fixatives, which can cause interference and contradiction in odorant analysis especially using mass spectrometry and human subject, are included in odorant samples. Moreover, we do not know the pure odorant mass spectrum. Therefore, it is essential to eliminate the interference of fixatives from the odor sample mass spectrum data and to extract the pure odor mass spectrum. In the present study, we performed independent component analysis (ICA) on the mass spectra of odor samples to remove the influence of fixatives. The advantage of the ICA in separating independent components without a priori knowledge of the original data is useful. The abundance of essential-oil mass spectra that we gathered were utilized as odorant samples. The results were compared with sensory test data from a human subject for a better study of fixative influence. It was revealed that ICA could extract the pure odor sample mass spectrum data without the influence of fixatives, even if the fixatives were added to the odor samples. This study’s outcomes allow us to analyze more odor samples for odorant analysis, not hindered by the influence of unwanted compounds.

INDEX TERMS Odorant analysis, odor samples, fixatives, ICA, independent components, correlation coefficient, sensory test.

I. INTRODUCTION
The interaction between an odorant and olfactory receptors leads to an odorant stimulus. This sensation is one of the primary senses and is an essential sense among living organisms. It plays an essential role in the behavior of living organisms, e.g., as a signal for potential danger, in the selection of food, in relaxation, and in finding mates [1]. Even though smell may be inferior to vision [2], olfactory stimuli develop a deeper subconscious response in humans [3]. It is known that humans can distinguish among up to 10,000 distinct odors with their known 400 types of olfactory receptors [4]–[6].

Currently, there are many studies about odorant analysis utilizing biomimetic odor sensing systems called e-noses. They are composed of electronic instruments that can detect, analyze, and recognize odors [7], [8]. There are some methods for odor detection and odor pattern analysis, such as arrays of chemical sensors, biosensors, and mass spectrometry (MS)-based electronic noses. The data extracted by e-noses were used for odorant analysis [9]–[12]. However, there are still technical limitations of the e-noses [8] compared to their counterpart of biological odor sensing.

Out of several methods of odor detection method, Mass spectrometry has the advantages in the selectivity and reproducibility in sensing different odors as electronic nose [13], [14] whereas its device size make it impossible to be mobile. Mass spectrometry has the capability to obtain hundreds of variables (mass-to-charge ratio, m/z fragments) within a single measurement with high reproducibility [15], [16]. Each fragment from the measurement result can be regarded as a sensor response, and the dimension of the data can be considered as a number of sensors. The pattern that can be observed in the distribution of fragments is also unique to its corresponding odorant. Moreover, ion fragments from MS measurements contain information about functional groups, which correlate with odor characters and quality.
Even though noise might be included in measurement data, it can be suppressed with proper preprocessing before mass spectrum data analysis. These properties give MS advantages in terms of higher reproducibility, fluidity, and selectivity compared to other odor sensors using biosensors or chemical sensors [8], [9], [11], [12].

We previously studied the quantification of mixtures using MS [17]. Since it was found that MS has a high capability of mixture quantification without a collinearity problem, it was extended to the exploration of odor components to cover a wide range of odors [17]–[20]. It was successful in approximating odors in an essential oil using odor components.

The raw materials used in odor-related samples for odorant analysis come from essential oils or synthetic aromatic molecules of natural origin [21]. Such odor samples are mostly composed of organic compounds, and they have limited shelf-life, while the odor quality also gradually diminishes over time or when exposed to certain conditions such as direct sunlight. To prevent this from happening, some chemical compounds are added to prolong the shelf-life of the odor samples. These chemical compounds are called fixatives. There are organic fixatives and synthetic fixatives; the former has an odorous characteristic, while the latter are mostly odorless and colorless [21]. The odorless characteristic of fixatives can cause unwanted interferences in odorant analysis, such as mass spectrometry. Specifically, these fixatives remain undetectable by the human nose, thereby causing a contradiction in odorant analysis.

We studied the mass spectrum data of essential oils available in our inventories [15], [16]. We explored and analyzed the odor samples using a large-scale mass spectrum database of essential oils. As mentioned before, odorless fixatives appear in the mass spectrum even though they remain undetectable by the human nose. This parasitic interference is considered as unwanted information during odor analysis using mass spectrum data. Thus, it is essential to identify the presence of odorless fixatives included in the mass spectrum data of odor samples before further odor analysis.

It is easy to quantify fixatives if the mass spectra of the pure odor sample and of the fixatives are known a priori. The linear least-squares method is sufficient to obtain the mixture composition in this case. However, the mass spectrum of pure odor samples is unknown in most cases even when the mass spectra of fixatives are known. Thus, we can obtain the mass spectrum of the pure odor sample after the intentional addition of fixatives to the sample in this situation followed by ICA analysis in this study.

II. CONCEPT OF PURE SAMPLE EXTRACTION

We considered odor samples and fixatives as different components that can be separated through mass spectrum data analysis. The idea of this study in suppressing the interference caused by the influence of fixatives and extract the data of pure odor sample is illustrated in Fig 1.

We applied independent component analysis (ICA) [22], [23] to the mass spectrum data to extract the independent components (ICs). The extracted independent components are mutually independent. Hence, the independency of the odor samples and fixatives is analyzed by identifying independent components corresponding to either odor sample of fixatives using Pearson’s correlation coefficient.

Randomly mixed data of odor samples and fixatives are analyzed using ICA. Then, an evaluation of the correlation between the extracted independent components and odor samples allows us to know whether fixatives are included in the odor sample. The extracted independent components are corresponding to either fixatives or odor sample. The independent component remaining after independent components of fixatives are identified corresponds to the one for pure odor sample. The independent component corresponding to the odorant sample is regarded as the pure odor sample mass spectrum shape which is truly unknown beforehand.

Furthermore, a sensory test with actual samples was also performed in this study. Samples with or without fixatives were used in the sensory test to reveal the characteristics of the fixatives. This was indispensable for validating the independent components extracted by ICA. Eventually, the success of this study will enable us to explore odorant
III. MATERIALS AND METHOD

A. SAMPLES

The samples used in this research is comprised of two groups. The first group consisted of odor samples, and the other group consisted of fixatives. The odor samples used in this study were 185 essential oils. Essential oil is a plant-derived aromatic oil that can be extracted using distillation, pressure, or hydro-diffusion [24]. Each essential oil has its own characteristic odor derived from the extracted plant. These characteristics vary depending upon geographical location, altitude, genetics, and extraction techniques.

Essential oils are widely used as a base for many odor samples in the perfumery, food, and cosmetic industries. Moreover, the essential oils also have a wide variety of uses for aromatherapy and medical use [24], [25]. We gathered essential oils from five companies (Absolute aromas, Zefir, Pranarom International, Palm Tree, and Naturas Psychos). Their essential oils are claimed to be pure or organic without synthetic materials. However, there is no information regarding the pure essential oils mass spectrum and the purity of essential oils. The complete list of essential oils is provided in Table S1 (Supplementary Materials). Examples of obtained essential oils are shown in Fig 3.

On the other hand, we evaluated five commonly used synthetic fixative samples with a nearly odorless characteristic in low vapor pressure and low volatility (benzyl benzoate, benzyl salicylate, propylene glycol, dipropylene glycol, and octanoic acid) [21]. The fixative samples were analytical-grade samples with a purity of at least 95%.

B. MASS SPECTROMETRY

The mass spectrometry data of odor samples (185 essential oils) and five fixatives were gathered. A mass spectrum is a fragment-ion pattern distribution. Each measured sample has its own characteristic mass spectrum distribution [10]. Mass spectra from the 50 m/z to 350 m/z region were gathered. Each sample was measured 10 times and diluted with ethanol before measurement. Since GC/MS is influenced by impurity of the solvent, ethanol with a purity of 99.5% was used for dilution. The dilution ratio for the measurement was 9:1 v/v (90% ethanol and 10% sample) [16]. The volume of sample injected into the GC/MS was 1 µL. Before and after injection of the samples, the syringe was washed five times with ethanol solvent. Since a lot of samples were measured, the dilution process and cleaning method were essential in preventing odor samples or fixatives from remaining in the measurement process.

An Agilent Technologies gas chromatograph/mass spectrometer (GC/MS, B5977 MSD coupled with 7890B GC) was used to measure the samples. A column without coating with an inner diameter of 0.1 mm and length of 10 m was used for GC (Agilent Technologies 160-2635-10; FS, Deactivated). Even though we bypassed the GC and only utilized the mass spectrometry data, the column could not be eliminated because of the pressure difference inside and outside the mass spectrometer. The carrier gas for the measurement was helium with a purity of 99.99995%. The detection time of the MS detector was set to 3 min. The remaining conditions, such as the temperature for the measurement process, are shown in Table 1. Furthermore, the measurement device, example mass spectra of essential oil and fixative, are shown in Figs 4a-c.

Typically, GC/MS needed an hour or more to complete a measurement [14], [26]. However, bypassing the GC enabled us to drastically shorten the measurement time compared to the situation when including GC part for the measurement procedure. Thus, each measurement took only 6–7 min, with 10 measurements completed in 60–70 min [16]. This automated procedure significantly reduced the labor and time needed to measure entire samples.
TABLE 1. GC/MS conditions.

| Variable               | Condition/Notes |
|------------------------|-----------------|
| Split ratio            | 1:100           |
| Injector temperature  | 250 °C          |
| Column temperature    | 250 °C          |
| Auxiliary port tempera| 230 °C          |
| Ion source temperature| 150 °C          |
| Ionization method     | Electronic ionization (70eV) |

C. INDEPENDENT COMPONENT ANALYSIS

Independent component analysis (ICA) is a data analysis technique that allows us to separate independent components (ICs) underlying the observed data. The underlying components are intended to be fully independent [22], [23], [27]–[29]. It is considered as a blind source separation (BSS) mathematical model [27]. It is used when the observed data is a linear mixture of signals from independent sources for the inputs and determines the transformation of the reconstruction back to the original one without any a priori information about the mixture. ICA does not require any supervision or prior stimulus. It has the ability to separate the independent components based on the stimulus given in the input; then, unsupervised learning is required. Here, let us denote linear mixture of the independent components by \( m \)-dimensional observed data of \( \mathbf{Y} = (y_1, y_1, \ldots, y_r) \in \mathbb{R}^{r \times m} \) and \( n \)-independent components by \( \mathbf{V} = (v_1, v_2, \ldots, v_n) \in \mathbb{R}^{n \times m} \), where \( r \) is the number of observed data. Furthermore, \( \mathbf{X} \in \mathbb{R}^{n \times r} \) demixing matrix for \( \mathbf{Y} \) to obtain \( \mathbf{V} \). The relationship of this problem is determined by:

\[
\mathbf{V} = \mathbf{X} \mathbf{Y}
\]

ICA usually recovers, at most, the same number of independent components as the number of observed data. Even though the number of mixed observed data can be increased, this leads to an extensive extraction process and time-consuming procedure. The number of independent components should be less than the number of observed data, and at most, same as the number of observed data. In our study, 100 randomly generated mixed observed data from each odor sample and fixative were analyzed using ICA. We utilized fastICA available in MATLAB software.

It is known that linear superposition is valid for the mass spectrum of a mixture. Thus, the data were generated computationally according to the random proportion and the linear superposition theorem. Furthermore, this procedure was repeated 100 times to investigate the performance of the ICA in extracting independent components from mixed observed data. Pearson’s correlation coefficient was utilized to identify ICs corresponding to either odor samples or fixatives.

D. SENSORY TEST

As mentioned before, the fixative samples used were nearly odorless and colorless. To confirm this, we separated samples into two groups for the sensory test. Group 1 contained samples without fixatives, and group 2 contained samples with fixatives. The primary goal of this sensory test was to evaluate the effect of fixatives on the odor characteristics of the odor samples.

We prepared two kinds of samples for this sensory test; Essential oil of organic orange sweet (Citrus aurantium) as sample A and essential oil of spearmint (Mentha spicata) as sample B. For the samples with fixatives, we added different proportions of fixatives to the orange and spearmint samples. All samples were diluted with ethanol with a ratio of 9:1. Further details about the proportion of fixatives in each odor sample are shown in Table 2. The fixative ratios...
were chosen from randomly generated data for ICA training. However, the ratios for the five fixatives mixed with organic orange sweet were equal, whereas those mixed with spearmint were in the ratio 1:1:3:2:1 (benzyl benzoate, benzyl salicylate, propylene glycol, dipropylene glycol, and octanoic acid, respectively) in the sensory test. The mass spectra of samples used in sensory test were measured as shown in Figs 5 a and b.

A sensory test by humans was performed for evaluation. A same/different test (or simple different test) was performed for sensory evaluation. The same/different sensory test is a test where a subject is requested to judge whether the provided pair of samples have the same or different odor. The smell was provided in a vial (Agilent Technologies 5182-0714; 2ml Vial) and the subject needed to sniff its smell. An illustration of the same/different test is shown in Fig 6. The provided smell was randomly given to subjects within the conditions given in Table 3. Each subject was provided with four pairs of odor samples including two pairs of samples A and B, but the pairs were randomly based on the conditions in Table 3. The McNemar test was performed for the evaluation of the sensory test result. A chi-square test would have been inappropriate for the evaluation procedure since each subject received both matched and unmatched pairs of samples. Thus, the number of tests was not equal to the number of subjects [30].

### IV. RESULTS

#### A. INDEPENDENT COMPONENT ANALYSIS RESULT

The mass spectral data of 185 essential oils and five fixatives were measured. Since small noise can appear in the measurement process, we applied preprocessing to the mass spectral data to eliminate the possible noise. This preprocessing procedure was done by searching for the coefficient of variation at the highest intensity ($CV(x_{\text{max}})$) in the averaged data $\mu$ at each $m/z$ ($x$) within measured 50 to 350 $m/z$ region of each sample. If the coefficient of variation of each $m/z$ ($CV(x)$) was higher than $CV(x_{\text{max}})+1\%$, it was regarded as noise, and its value was replaced with zero [16]. Then, we applied ICA to the preprocessed data. The preprocessing equation is as follows:

$$x_{\text{max}} = \arg \max_{50 \leq x \leq 350} \mu(x)$$  \hspace{1cm} (2)

$$\mu(x) = \begin{cases} 0, & \text{if } CV(x) > CV(x_{\text{max}}) + 1\% \\ \mu(x), & \text{if } CV(x) < CV(x_{\text{max}}) + 1\% \end{cases}$$  \hspace{1cm} (3)

Each iteration of ICA was performed with 100 artificially generated data from the mixture of an odor sample and the five fixatives in a numerical simulation. The mass spectra of the odor samples, five fixatives, and mixed observed data are shown in Fig 7. ICA was provided with information on the number of odor sample and fixatives prior to the training. Here, ICA was performed to identify six independent components corresponding to either odor sample or the five fixatives included in the mixed observed data. This procedure was repeated 100 times to determine the stability of ICA in finding independent components.

Basically, ICA cannot identify the independent components in order. Thus, we applied Pearson’s correlation coefficient to identify each IC. The result of ICA for the odor sample of orange sweet (Citrus aurantium) and five fixatives is shown in Table 4 as an example.

We calculated the correlation coefficient between each IC and the mass spectrum of odor sample/fixatives.
First, independent components corresponding to five fixatives are identified using a correlation coefficient. Then, the remaining independent component can be regarded as a counterpart of the pure odor sample. Here, the highest correlation coefficient in each column indicates the IC corresponding to the odor sample or fixative in the row. For example, IC 2 had the highest correlation coefficient with orange sweet (0.98). It means that IC 2 has a very strong correlation with orange sweet [31]; we regarded IC 2 as orange sweet. The other ICs also had a very high correlation with other specific samples. The results of ICA are plotted in Figs 8a–g. The IC and corresponding odor sample or fixative are plotted in the same figure (Figs 8b–g) for easier understanding. It was proven that the patterns of IC 2 and orange sweet are close to each other (Fig 8b), as well as those of the other ICs and their corresponding fixatives.

![FIGURE 7. Mass spectra of original data (top) and generated mixed observed data prepared for ICA (bottom). Only the first nine mixed observed data are shown here.](image)

The correlation coefficient was used not only to identify ICs corresponding to the odor sample or fixatives but also to determine the presence of fixatives in the odor sample. Odor sample is pure one if the correlation coefficient is close to one. The fixatives are included in the odor sample if the correlation coefficient is low. The row of orange sweet in Table 4 other than IC 2’s element can be used to determine the presence of fixatives in the orange sweet. First, focus on the correlation of IC 2 and orange sweet with a value of 0.98. Then see the correlation coefficient of other ICs with orange sweet. Therefore, we can say that the IC 2 independently correlates to orange sweet and is mutually independent to other ICs. Hence IC 2 solely corresponds to orange sweet, and orange sweet does not contain any fixatives included in our study.

In the case of fixatives, the benzyl benzoate results showed the highest correlation coefficient with IC 6; however, its correlation coefficient with IC 1 was also relatively high (0.51) despite IC 1 corresponding to benzyl salicylate (0.99). This indicates that benzyl benzoate is not truly independent and partially overlaps with benzyl salicylate (see Figs 8c, d). Hence, we evaluated the correlation coefficients of each fixative as shown in Fig 9, indicating a moderate correlation between benzyl benzoate and benzyl salicylate (0.46). Thus, the above-described overlap between benzyl benzoate and IC 1 is reasonable.

We evaluated the performance of ICA for all 185 odor samples. The average correlation coefficient between ICs and their corresponding odor sample for all trained data was 0.945, with a standard deviation of 0.0687. The highest correlation coefficient was 0.999, and the lowest was 0.674, respectively. Their distribution is shown in Fig 10 and the result with respective parameter shown in Table 5. Since we obtained high correlation coefficients between odor samples and their corresponding ICs, we can accordingly obtain the mass spectra of pure odor samples from their corresponding ICs. We evaluated a p-value of 0.0016 for the results with an intended correlation coefficient of 1, suggesting no significant presence of fixatives (p < 0.05) in the odor sample as a function of the analysis of independent components obtained through ICA.

Even though the statistical data of overall 185 odor samples shows that the odor samples are indeed pure, we extracted the result of the odor sample with lowest correlation coefficient, which was Chamaecyparis obtus endl (0.674), for further analysis. Again, we calculated the correlation coefficient between each IC and the mass spectrum of odor sample/fixatives and shown in Table 6.

Here, IC 1 regarded as IC that corresponds to Chamaecyparis obtus endl (0.67) since other ICs correspond strongly to the fixatives. However, Chamaecyparis obtus endl also has a moderately high correlation coefficient with other ICs, such as IC 2 (0.7), even though IC 2 solely corresponds to benzyl benzoate. Thus, it can be concluded that the fixative of benzyl benzoate is included within the odor sample of Chamaecyparis obtus endl.

Moreover, IC 1 which corresponds to the Chamaecyparis obtus endl, does not have a strong correlation with other fixatives. Hence, we regarded IC 1 as pure mass spectra of Chamaecyparis obtus endl after the removal of the influence of fixatives. The result of Table 6 is different from Table 4, where the odor sample of orange sweet correlates only to IC 2. The mass spectra comparison of the odor sample and its corresponding IC 1 is shown in Fig 11. The mass spectrum shows the similarity of pattern as well as suppressed several peaks of Chamaecyparis obtus endl in the IC 1. Hence, the fixatives influence was indeed suppressed in the IC 1.

Moreover, we generated mass spectral data of 185 odor samples with the intentional addition of fixatives. We created a new dataset of randomly generated mixed observed data using each odor sample containing fixatives and the five fixatives. ICA was performed with this dataset. Then, we evaluate the correlation coefficient between ICs and their corresponding odor samples. An average correlation coefficient of 0.629 (0.604–0.71) was obtained, which is lower than the correlation coefficient of odor sample without fixatives in Fig 10; their distribution is shown in Fig 12 and the result with respective parameter is shown in Table 7. This result
indicates that including fixatives in the odor samples indeed decreased the correlation to their corresponding independent components. The odor samples with fixatives correlated not only to one independent component but also to other independent components that corresponded to the fixatives. The decrease is the correlation coefficient is reasonable and we moved to the next experiment.

Furthermore, we evaluated the correlation coefficients between ICs corresponding to odor samples (with fixatives added) obtained before and the original odor samples without fixatives. We obtained an average correlation coefficient result of 0.949 (0.704–0.99). The result is shown in Fig 13 and Table 8. These result almost agrees with Fig 10 as well as Table 5, where odor samples without fixatives were used to generate the mixed observed data. Thus, one can conclude that ICA can extract ICs corresponding to pure odor samples without the influence of fixatives despite the presence of fixatives in the odor samples.
TABLE 4. The correlation coefficient between original data and independent components (ICs) in orange sweet odor sample.

|       | IC1   | IC2   | IC3   | IC4   | IC5   | IC6   |
|-------|-------|-------|-------|-------|-------|-------|
| Orange sweet | 0.19  | 0.98  | 0     | 0.01  | 0     | 0.07  |
| Benzyl benzoate | 0.51  | 0.01  | 0     | 0.01  | 0.01  | 0.86  |
| Benzyl salicylate | 0.99  | 0.03  | 0     | 0.01  | 0.01  | 0.06  |
| Propylene glycol | 0    | 0     | 0.1   | 0.99  | 0.05  | 0     |
| Dipropylene glycol | 0.01  | 0.01  | 0.99  | 0.02  | 0.03  | 0     |
| Octanoic Acid | 0.01  | 0.01  | 0.02  | 0.1   | 0.99  | 0.01  |

B. RESULT OF SENSORY TEST

One concern was that the balance among ingredients in a sample in the gas phase might be influenced by fixatives even if the fixatives are odorless. Thus, a sensory test was conducted to evaluate the influence of the fixatives in odor samples’ odor characteristics. A same/different test was conducted with two different odor samples (orange sweet and spearmint). The participants were 15 nonexperts aged between 22 and 51 years old (females and males). The subjects were only exposed to sample pairs once.

The sensory evaluations for the orange sweet and spearmint are shown in Tables 9 and 10, respectively. The conditions to which the subjects were exposed corresponded to Table 3, and two pairs of orange sweet and spearmint were given to each subject. They aimed to determine whether the given pair had the same smell or a different smell before answering on the given sheet. Since there were no color differences between samples with and without fixatives, the sensory test was not conducted with a blindfold for the subject. The sensory test was conducted in the room with sufficient airflow to prevent the smell from lingering in the air. This was essential to prevent the bias of judgment caused by the previous sensory test.

**FIGURE 9.** Correlation coefficients between fixatives.

We calculated McNemar’s $T$ using the sensory test results for the evaluation as follows [30]:

$$\text{McNemar’s } T = \frac{(w - y)^2}{w + y} \quad (4)$$

$T$ is rejected if its value is greater than the critical value of chi-square [30]. This sensory test had one degree of freedom, and we empirically selected a $p$-value $<0.05$ for the evaluation. Hence, if a $T$ value greater than chi-square $1.0,0.05 = 3.84$, would suggest that the samples are different. The $T$ values for the sensory test result of orange sweet and spearmint were 1.8 and 0.111, respectively. Both values were smaller than the critical value of 3.84; therefore, it can be concluded that there was no difference in smell between the samples with and without fixatives in the sensory test.

V. DISCUSSION

Essential oils, which are used as the base of many odor samples, are widely available on the market. However, most of them do not include a list of ingredients, and the actual purity of the products is not known, and we do not know the mass spectrum of pure one a priori. On the other hand, other odor samples, such as perfumes, mostly have an odor concentration less than 20 vol.%. Even eau de cologne perfume has only up to 4 vol.% odor concentration in 70–85 vol.% ethanol [21]. Since most odors have odorless chemical compounds, a method that allows us to extract only those compounds that correlate to the odor for odorant analysis is essential. Each chemical compound has its own specific molecular weight and mass. Thus, it has its own characteristic mass spectrum regardless of odor their odor characteristics.

This paper studies the use of ICA in the suppression of influence of odorless fixatives although ICA is a well-known method used in audio mixture analysis, electrocardiogram analysis, and image data analysis, as mentioned in the referenced papers. The novelty in the field of machine olfaction here is the use of ICA in suppressing the influence of odorless fixatives. When an odor sample is measured using mass spectrometry (MS), the influence of fixatives appears in the measurement result. However, humans cannot detect the smell of fixatives in odor samples with or without fixatives (Fig 5a,b). This difference can cause contradictions; thus,
TABLE 6. The correlation coefficient between original data and independent components (ICs) in chamaecyparis obtus endl odor sample.

| IC     | IC1 | IC2 | IC3 | IC4 | IC5 | IC6 |
|--------|-----|-----|-----|-----|-----|-----|
| Chamaecyparis obtus endl | 0.67 | 0.7 | 0.05 | 0.15 | 0.13 | 0.16 |
| Benzyl benzoate | 0.2 | 0.83 | 0.01 | 0.52 | 0 | 0 |
| Benzyl salicylate | 0.11 | 0.05 | 0.01 | 0.99 | 0 | 0 |
| Propylene glycol | 0 | 0 | 0.05 | 0 | 0.99 | 0.1 |
| Dipropylene glycol | 0.01 | 0 | 0.03 | 0 | 0.02 | 0.99 |
| Octanoic Acid | 0.03 | 0 | 0.99 | 0.01 | 0.1 | 0.02 |

FIGURE 11. Mass spectrum Chamaecyparis obtus endl and corresponding IC 1 showing agreement of pattern as well as suppressed peak which regarded as fixatives.

Removing the influence of fixatives for odorant analysis is essential.

Even though mass spectrometry has advantages in providing high-dimensional data and other features [8], [13], it cannot separate the true mass spectrum of an odor sample from unwanted compounds included in the measurement by itself. If an odor sample includes fixatives, its mass pattern is a combination of the patterns of pure odor and the fixatives. We could not separate the fixatives without information of mass spectrum of pure odor sample when the conventional analysis method is used.

Despite not knowing the proportion of fixatives within the sample, ICA can extract the independent components corresponding to either odor sample or fixatives according to our study. We treated the independent components corresponding to odor sample as the pure shape of odor sample mass spectrum since it is mutually independent to other ICs (other ICs correspond to fixatives).

Mass spectrometry allowed us to provide the ICA with a sufficient amount of data and prevented ICA from underfitting when identifying independent components. Furthermore, the proportions of fixatives or odor samples when reconstructing the mixed observed data were represented by the magnitude of the demixing matrix in the ICA. This means that, even though the observed mixed data were changed, the independent component vectors remained the same, despite the magnitude of the demixing matrix changing to compensate for the change in the mixed observed data.

TABLE 7. The correlation coefficient result between odor sample (with fixatives within) with corresponding ICs in respective parameter (average of 185 samples).

| Parameter | Result |
|-----------|--------|
| Average of 185 sample’s correlation coefficient | 0.629 |
| Highest correlation coefficient | 0.71 |
| Lowest correlation coefficient | 0.604 |
| Standard deviation | 0.017 |

FIGURE 12. Charts of correlation coefficients between 185 odor samples with fixatives intentionally added and their respective ICs The plot is sorted from highest to lowest. Average value from 100 times trial from each odor sample are shown.

The ability of ICA in the extraction of independent components was further evaluated when odor samples were intentionally mixed with fixatives beforehand. We evaluated the ICA results from 185 odor samples. ICA allowed us to extract the independent components without the influence of fixatives, even if odor sample data was intentionally mixed with...
fixatives beforehand (Fig 12 and Fig 13). Furthermore, the sensory test conducted in our study also revealed that the odorless fixatives did not have any influence on the odor characteristics of samples. The same result was obtained despite carrying out the sensory test for different samples with different ratios of essential oils and fixatives. Accordingly, it was found that ICA could extract the true odor sample’s mass spectrum in spite of the presence of fixatives or other chemical compounds without odorant characteristics. It is emphasized that the pure mass spectrum of an odor sample can be extracted using ICA when many data intentionally mixed with interfering mass spectra at random were used.

**TABLE 9.** Same/different test result for odor sample of orange sweet.

| Subject received A1/A2 or A2/A1 and responded | Same | Different |
|-----------------------------------------------|------|-----------|
| Subject received A1/A1 or A2/A2 and responded  | $x = 17$ | $w = 4$ |
|                                                | $y = 5$ | $z = 4$ |

**TABLE 10.** Same/different test result for odor sample of spearmint.

| Subject received B1/B2 or B2/B1 and responded | Same | Different |
|-----------------------------------------------|------|-----------|
| Subject received B1/B1 or B2/B2 and responded  | $x = 21$ | $w = 1$ |
|                                                | $y = 4$ | $z = 4$ |

Despite the various advantages of ICA in identifying independent components, a disadvantage may occur when analyzing the mass spectral data with ICA. Mass spectra have a non-negativity property, whereas negative values might appear in ICA after training. Although we tried to improve the problem here by adding nonnegative constraints during training, this may have decreased the accuracy of ICA. However, this procedure is indispensable for odorant analysis using mass spectrometry.

The introduction of ICA into mass spectrum data analysis to extract odor samples data without the influence of fixatives are significant when we want to increase the number of odorant samples for odorant analysis. Upon gathering enough mass spectral data of fixatives, the separation of an odor sample’s pattern from that of its fixatives is possible. A remaining concern is the unavailability of ingredient information in some odor samples. Thus, we might need to gather data from the fixatives used in other odor samples. In the end, as long as enough data are provided on chemical compounds used in particular odor samples, we might explore the pure odor sample’s data separated from unwanted parasitic interferences when using ICA. Nevertheless, further studies exploring samples for odorant analysis are still needed.

**VI. CONCLUSION**

We demonstrated that ICA allows extracting independent components corresponding to the pure original odor regardless of the presence of fixatives with high correlation in the mass spectrum space. Furthermore, a sensory evaluation also revealed the odorless property of fixatives according to McNemar’s T value. Thus, this study opens the way to explore more odor sample for odorant analysis using mass spectrometry data space without the concern of interferences caused by unwanted chemical compounds.

**REFERENCES**

[1] T. C. Pearce, S. S. Schiffman, H. T. Nagle, and J. W. Gardner, Eds., “Handbook of machine olfaction,” in Handbook of Machine Olfaction. Hoboken, NJ, USA: Wiley, 2002.
[2] M. Meister, “On the dimensionality of odor space,” eLife, vol. 4, Jul. 2015, Art. no. e07865, doi: 10.7554/eLife.07865.
[3] A. Rinaldi, “The scent of life: The exquisite complexity of the sense of smell in animals and humans,” EMBO Rep., vol. 8, no. 7, pp. 629–633, Jul. 2007, doi: 10.1038/sj.embor.7401029.
[4] L. Buck and R. Axel, “A novel multigene family may encode odorant receptors: A molecular basis for odor recognition,” Cell, vol. 65, no. 1, pp. 175–187, Apr. 1991, doi: 10.1016/0092-8674(91)90418-X.
[5] B. Malnic, P. A. Godfrey, and L. B. Buck. (2004). The Human Olfactory Receptor Gene Family. Accessed: Apr. 1, 2021. [Online]. Available: www.pnas.org/cgi/10.1073/pnas.0307882100.
[6] C. Bushdid, M. O. Magnasco, L. B. Voshall, and A. Keller. “Humans can discriminate more than 1 trillion olfactory stimuli,” Science, vol. 343, no. 6177, pp. 1370–1372, Mar. 2014, doi: 10.1126/science.1249168.
[7] H. Nanto and J. R. Setter, “Introduction to chemosensors,” in Handbook of Machine Olfaction, T. C. Pearce, S. S. Schiffman, H. T. Nagle, and J. W. Gardner, Eds. FRG: Weinheim, Germany, 2003, pp. 79–104.
[8] F. Röck, N. Barsan, and U. Weinmar, “Electronic nose: Current status and future trends,” Chem. Rev., vol. 108, no. 2, pp. 705–725, Jan. 2008, doi: 10.1021/cr068121g.
[9] T. Dung, Y. Oh, S.-J. Choi, I.-D. Kim, M.-K. Oh, and M. Kim, “Applications and advances in bioelectronic noses for odor sensing,” Sensors, vol. 18, no. 2, p. 103, Jan. 2018, doi: 10.3390/s18010103.
[10] T. Nakamoto, M. Ohno, and Y. Nihei, “Odor approximation using mass spectrometry,” IEEE Sensors J., vol. 12, no. 11, pp. 3225–3231, Nov. 2012, doi: 10.1109/JSEN.2012.2219050.
[11] R. Kanazaki, K. Nakatani, T. Sakurai, N. Misawa, and H. Mitsuno, “Physiology of chemical sense and its biosensor application,” in Essentials of Machine Olfaction and Taste, T. Nakamoto, Ed. Singapore: Wiley, 2016, pp. 3–48.
[12] M. Bernabei, S. Pantalei, and K. C. Persaud, “Large-scale chemical sensor arrays for machine olfaction,” in Essentials of Machine Olfaction and Taste, T. Nakamoto, Ed. Singapore: Wiley, 2016, pp. 49–86.
[13] E. Llobet, O. Gualdrón, M. Vinaixa, N. El-Barbri, J. Brezmes, X. Vilanova, B. Bouchikhi, R. Gómez, J. A. Carrasco, and X. Correig, “Efficient feature selection for mass spectrometry based electronic nose applications,” Chemometric Intell. Lab. Syst., vol. 85, no. 2, pp. 253–261, Feb. 2007, doi: 10.1016/j.chemolab.2006.07.002.
[14] M. Delgado-Rodríguez, M. Ruiz-Montoya, I. Giraldez, R. López, E. Madejón, and M. J. Díaz, “Use of electronic nose and GC-MS in detection and monitoring some VOC,” Atmos. Environ., vol. 51, pp. 278–285, May 2012, doi: 10.1016/j.atmosenv.2012.01.006.
[15] D. Prasetyawan and T. Nakamoto, “Comparison of NMF with Kullback–Leibler divergence and Itakura-saito divergence for odor approximation,” in Proc. IEEE Int. Symp. Olfaction Electron. Nose (ISOEN), May 2019, pp. 1–3, doi: 10.1109/ISOEN.2019.8823186.
[16] D. Prasetyawan and N. Takamichi, “Sensory evaluation of odor approximation using NMF with Kullback–Leibler divergence and Itakura–Saito divergence in mass spectrum space,” J. Electrochem. Soc., vol. 167, no. 16, Dec. 2020, Art. no. 167520, doi: 10.1149/1945-7116/abd110.
[17] T. Miura, T. Nakamoto, and T. Moriizumi, “Study of odor recorder using mass spectrometry,” in Proc. IEEE Virtual Reality Conf., Mar. 2009, pp. 159–162, doi: 10.1109/VR.2009.4811016.
T. Nakamoto and Y. Nihei, “Improvement of odor approximation using mass spectrometry,” IEEE Sensors J., vol. 13, no. 11, pp. 4305–4311, Nov. 2013, doi: 10.1109/JSEN.2013.2267728.

T. Nakamoto, “Olfactory display and odor recorder,” in Essentials Machine of Olfaction and Taste. Singapore: Wiley, 2016, pp. 247–314.

M. vom Ende, W. Sturm, and K. Peters, “Perfumes,” in Ullmann’s Encyclopedia of Industrial Chemistry. Hoboken, NJ, USA: Wiley-VCH Verlag GmbH & Co. KGaA, 2017, pp. 1–7.

J.-X. Mi, “A novel algorithm for independent component analysis with reference and methods for its applications,” PLoS ONE, vol. 9, no. 5, May 2014, Art. no. e93984, doi: 10.1371/journal.pone.0093984.

A. Hyvärinen, “Independent component analysis: Recent advances,” Phil. Trans. Roy. Soc. A, Math., Phys. Eng. Sci., vol. 371, no. 1984, Feb. 2013, Art. no. 20110534, doi: 10.1098/rsta.2011.0534.

C. R. Manion and R. M. Widder, “Essentials of essential oils,” Amer. J. Health-Syst. Pharmacy, vol. 74, no. 9, pp. e153–e162, May 2017, doi: 10.2146/ajhp151043.

F. Firenzuoli, V. Jaitak, G. Horvath, I. H. N. Bassolé, W. N. Setzer, and L. Gori, “Editorial essential oils: New perspectives in human health and wellness,” Evidence-Based Complementary Alternative Med., vol. 2014, Jun. 2014, Art. no. 467363, doi: 10.1155/2014/467363.

P. Bruno, M. Caselli, G. de Gennaro, M. Solito, and M. Tutino, “Monitoring of odor compounds produced by solid waste treatment plants with diffusive samplers,” Waste Manage., vol. 27, no. 4, pp. 539–544, Jan. 2007, doi: 10.1016/j.wasman.2006.03.006.

T. Isomura and T. Toyozumi, “A local learning rule for independent component analysis,” Sci. Rep., vol. 6, no. 1, pp. 1–17, Jun. 2016, doi: 10.1038/srep28073.

A. Hyvärinen, J. Hurri, and P. O. Hoyer, Independent Component Analysis. London, U.K.: Springer, 2009, pp. 151–175.

I. Goodfellow, Y. Bengio, and A. Courville, “Linear factor models,” in Deep Learning, I. Goodfellow, Y. Bengio, and A. Courville, Eds. Cambridge, MA, USA: MIT Press, 2016, pp. 485–498.

M. C. Meilgaard, G. V. Civille, and B. T. Carr, “Overall difference tests: Does a sensory difference exist between samples?,” in Sensory Evaluation Techniques, 5th ed. Boca Raton, FL, USA: CRC Press, 2016, pp. 79–122.

R. Taylor, “Interpretation of the correlation coefficient: A basic review,” J. Diagnostic Med. Sonogr., vol. 6, no. 1, pp. 35–39, 1990, doi: 10.1177/875647939000600106.

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