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## RESEARCH ARTICLE

# Sensory Evaluation of Interference Suppression in Odor Reproduction Using Mass Spectrometry

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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 a contradiction in odorant analysis especially between 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** Mass spectrometry, odorant analysis, odorant samples, sensory evaluation, ICA, independent components, fixatives.

## I. INTRODUCTION

Odorants can stimulate olfactory receptors that can lead to a sense of smell in living things. The smell sense is considered as one of the primary senses and an essential part of the survival of living organisms [1]. The human sense of smell is a complex system and is widely known to have around 400 types of olfactory receptors and discriminate up to 10,000 different odorants [2], [3], [4], [5].

Studies regarding odorant analysis are still ongoing. These studies mimicking human odor-sensing system is often called electronic noses or e-nose. An e-nose can perform odor detection, analysis, and recognition [4], [6]. Different kind of

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sensors can be utilized by e-noses in the detection of odorants. Chemical sensors, biosensors, gas chromatography (GC), and mass spectrometry (MS) are widely used as odor detection sources [4], [6], [7], [8], [9], [10], [11], [12], [13]. Even though we have different kinds of sensors that can be used for odor detection, they are still inferior to complex biological odor sensing [4], [6].

Several researchers utilized mass spectrometry for the odor detection method due to its advantages in sensitivity, reproducibility, and stability during odorant sensing. Moreover, mass spectrometry also enables us to detect hundreds of variables in the range of mass-to-charge ratio ( $m/z$  fragments) that resemble an array of sensors within a single measurement with high reproducibility [6], [8], [11], [12]. Each detected single fragment could be regarded as a sensor response.

Hence, the detected range of  $m/z$  fragments can be considered as the number of sensors, i.e., the dimension of the data. These detected fragments can have a pattern that is unique to the corresponding odorant. Furthermore, mass spectrometry measurement also contains information regarding functional groups that contribute to the characteristic and quality of odorants. However, noise also can appear during the measurement of MS. The noise can be suppressed with proper preprocessing before mass spectrum data analysis.

In our previous study, we found that MS has a high capability of mixture quantification without collinearity problems [8], [14]. Therefore, we extended our study to the odor components exploration that covers a wide range of odors. As a result, odorant approximation of essential oils was successfully performed with odor components [8], [14], [15].

Odor sense does not have a set of primary components as color in vision. Despite the effort to find primary odors, there were still limitations, such as the number of samples utilized for odor reproduction, and the exact number of primary odors is still unknown [7], [8], [13], [16].

However, increasing the number of odorant samples for odor reproduction proves to be difficult due to the appearance of unwanted compounds such as preservatives in odorant samples. Since odor samples have limited shelf-life time, adding additional chemical compounds to extend the shelf-life of odorant samples is common. These chemical compounds are often called fixatives. There are fixatives originating from natural sources and synthetic ones. Natural fixatives still have the odorous characteristic, but synthetic ones are odorless [17].

The odorless synthetic fixatives cause interference during odorant analysis. Although they are undetected by the human nose due to their odorless property, synthetic fixatives can be detected by mass spectrometry. Hence it can cause a contradiction in the odorant analysis. In most cases, we do not know the pure odorant mass spectrum without fixatives despite the knowledge of the fixatives' mass spectrum. Thus, we can obtain the mass spectra of pure odorant samples with the addition of fixatives to the sample, followed by applying independent component analysis (ICA) in this study. ICA was successfully utilized to extract independent components (ICs) that correspond either to mass spectra of fixatives or pure odorant samples [18]. However, we only have the numerical analysis result. Therefore, the sensory test is indispensable for evaluating the result from ICA.

In this study, we performed odor approximation as odorant analysis with physical odorant samples followed by sensing evaluation. Its workflow is shown in Fig 1. Here, we performed ICA on mixed samples to extract pure essential oils (extracted essential oils) separated from the fixatives. Then we compared the result of approximated odors from both original essential oils and extracted essential oils. The comparison was performed numerically as well as physical samples. The outcome of this study would confirm the performance of suppression of interference in odorant samples using sensory evaluation.

## II. MATERIALS AND METHOD

### A. SAMPLES AND MASS SPECTROMETRY

In this study, two groups of samples consisted of pure samples and mixed samples were prepared. Pure samples consisted of odorant samples and fixatives. 180 essential oils as odorant samples and five commonly used fixatives were prepared. Essential oil is an aromatic oil derived from a plant which can be extracted using distillation, pressure, or hydro-diffusion [19].

We gathered as many different types of essential oils as possible with various characteristics of smells. Therefore, each essential oil that we gathered has its own characteristic type of smell. We gathered essential oils from four different companies (Absolute Aromas, Zefir, Pranarom International, and Naturas Psychos). The complete list of essential oils used in this study is provided in Table S1.

Meanwhile, five different types of synthetic fixatives that we gathered were comprised of benzyl benzoate, benzyl salicylate, propylene glycol, dipropylene glycol, and octanoic acid. They are considered as synthetic fixatives with nearly odorless characteristics [17]. Those fixatives can change the vapor pressure and volatility of the odorant sample when mixed with the odorant sample with the aim of prolonging the shelf-life of the odorant sample. All fixatives we gathered were analytical-grade samples with a purity of at least 95%

On the other hand, each mixed sample was comprised of essential oil mixed with five fixatives. We prepared 180 samples mixed with essential oils and fixatives at different ratios. The ratio of essential oil and fixatives for each mixed sample is provided in Table S2. The mixing ratio to create mixed samples was obtained by generating a pseudorandom number generator.

This study gathered mass spectrometry data of 180 original essential oils, five fixatives, and 180 mixed samples. The data dimensionality was 201 since mass spectra from 50  $m/z$  to 250  $m/z$  regions was gathered from each sample. Mass spectrometry data is data that contains fragment-ion pattern distribution. Each sample has its own characteristic of mass spectrometry data [8], [15]. Each sample needed to be diluted with ethanol (99,5% purity) with a dilution ratio of 9:1 v/v (90% ethanol and 10% samples). In this study, each original essential oil and fixative were measured ten times. However, mixed samples were measured only five times. The reduction of measurement times was necessary to prevent fixatives from remaining in the measurement system.

Agilent Technologies gas chromatograph/mass spectrometer (GC/MS, B5977 MSD coupled with 7890B GC) was utilized to measure the samples. 1  $\mu\text{L}$  of the sample was injected into the GC/MS for measurement. Prevention of odor remaining in the measurement system was done by washing the syringe five times with ethanol before and after each measurement. Since this study was utilizing samples related to smell, preventing odor samples or fixatives from remaining within the measurement process becomes essential. If any samples remain within the measurement system, it will affect the measurement of other samples that are going

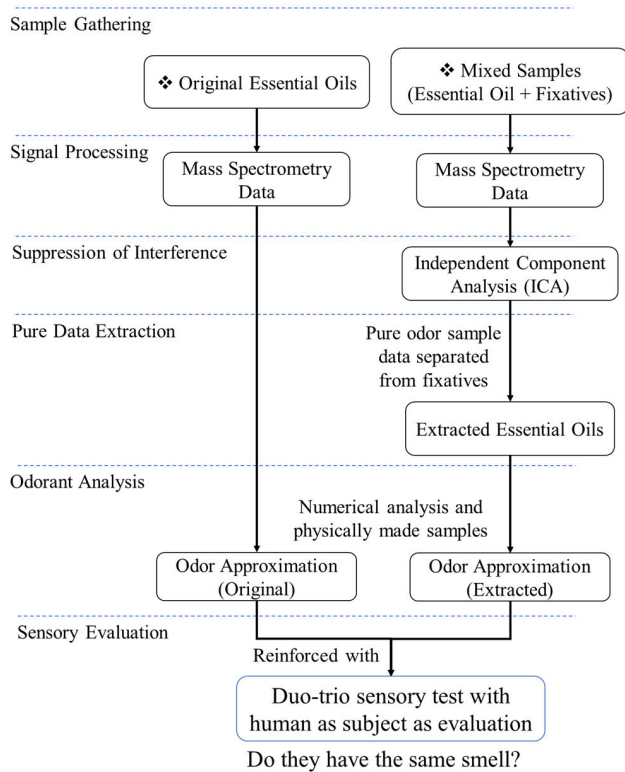


FIGURE 1. The workflow of the study.

to be measured. Examples of the gathered mass spectrum are shown in Figs 2a and b.

TABLE 1. GC/MS conditions.

Variable	Condition/Notes
Split ratio	1:100
Carrier gas	Helium (99.99995%)
Injector temperature	250 °C
Column temperature	250 °C
Auxiliary port temperature	230 °C
Ion source temperature	150 °C
MS detection time	3 minutes
Ionization method	Electronic ionization (70eV)

Specification of GC/MS utilized in the study is shown in Table 1. Although we only utilized mass spectrometry data and bypass the GC part, the column could not be eliminated because we needed to maintain pressure difference between the inside and outside of the mass spectrometer. Thus, a column (inner diameter 0.1 mm, length 10 m) without coating was utilized. Each measurement took 6 to 7 minutes to measure a sample. Typically, GC/MS needed hours to complete a single cycle of measurement [11]. In our setup, we needed only 60-70 minutes to measure ten times measurement and 30-35 minutes for the five times measurement. Hence, these GC/MS setups significantly reduced the labor and time we needed to measure entire samples [11], [12].

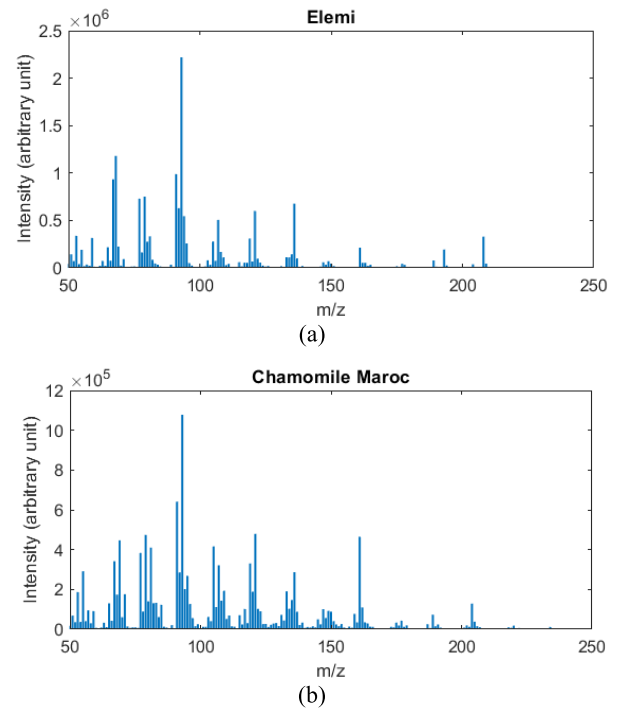


FIGURE 2. Example of essential oils mass spectrum gathered by GC/MS for (a) elemi and (b) chamomile maroc.

## B. INTERFERENCE SUPPRESSION

In this study, independent component analysis (ICA) was utilized for suppression of interferences. ICA can separate independent components (ICs) that are underlying within the observed data. ICA can be utilized well when observed data is a linear mixture of independent signals. ICA determines the transformation of the reconstruction of original data without any a priori knowledge about the mixture [20], [21]. In other words, ICA does not require any kind of supervision in separating observed data into independent components. Although PCA can be also used for dimensional reduction, ICA is more appropriate here due to its capability of the separation of mutually independent fixatives and pure sample. Here, let us denote a linear mixture of  $m$ -dimensional observed data by mass spectrometry  $\mathbf{Y} = (\mathbf{y}_1, \mathbf{y}_1, \dots, \mathbf{y}_d)$   $\mathbf{Y} \in \mathbf{R}^{d \times m}$ ,  $m$ -dimensional ICs of  $\mathbf{V} = (\mathbf{v}_1, \mathbf{v}_2, \dots, \mathbf{v}_n)$   $\mathbf{V} \in \mathbf{R}^{n \times m}$ , and  $\mathbf{X}$  ( $\mathbf{X} \in \mathbf{R}^{n \times d}$ ) demixing matrix for  $\mathbf{Y}$  to obtain  $\mathbf{V}$ , where  $d$  is the number of observed data of the mixture composed of sample and fixatives which is set to 100 in this study, and  $n$  is the number of ICs and set to six (five fixatives and one pure sample). Their relationship is determined by:

$$\mathbf{V} = \mathbf{X}\mathbf{Y} \quad (1)$$

Hence, in this study, ICA performed the separation of pure odorant samples from fixatives by analyzing mixed samples.

Here, we regarded fixatives as interference that can be detected by mass spectrometry. However, the human nose cannot detect them [18]. Thus, it causes a contradiction in the odorant analysis and should be omitted before odorant

analysis is performed. The main difference between the previous study [18] and this study is the form of mixed samples. In our previous study, large-scale mass spectrum data of mixed samples were generated artificially in the numerical form, and ICA was applied to it. However, in this study, the large-scale mass spectrum data of mixed samples were created physically and gathered using MS, and ICA was applied to it. In addition, FastICA, available in MATLAB software, was utilized in our study [22]. The symmetric algorithm with gaussian non-linearity function were utilized for the fastICA.

ICA can extract ICs at most, the same number as observed data. In this study, ICA was applied to 100 mixed samples data. First, 100 data was generated numerically with mixed samples by injecting additional fixatives data in it. Hence, ICA does not have any clue regarding the original data of essential oils since the mixed samples themselves were mixtures of essential oils and fixatives. Then, ICA extracted ICs which correspond to either fixatives or pure odor sample. Identification of ICs corresponding to either fixatives or pure odor sample was done by calculating the Pearson correlation coefficient. From here on, the pure odor samples extracted by ICA after analyzing mixed samples will be called as extracted essential oils.

### C. ODORANT ANALYSIS

Odorant analysis performed in this study is odor reproduction. Odor reproduction is a method to reproduce a target odor by blending a set of odor components. This reproduced odor is called as approximated odor. Different kinds of approximated odor can be produced by changing the blending ratio of odor components. Importantly, the approximated odor should be as close as possible to the target odor [8], [13], [14], [15].

In this study, nonnegative matrix factorization (NMF) was utilized to calculate the blending ratio and odor components for odor reproduction in numerical form. Particularly NMF with Itakura-Saito divergence (NMF-IS) [15]. NMF itself is a dimensional reduction technique that factorizes original data into two matrices. The size of factorized matrices is determined by the number of basis vectors [23]. NMF-IS was applied to large-scale mass spectrum data of original essential oils and another one to large-scale mass spectrum data of extracted essential oils to extract odor components.

Here, let us have a large-scale mass spectrum data of essential oils of matrix  $\mathbf{Q}$  with the size of  $k \times m$ , where  $k$  is the number of essential oils (180 essential oils). This matrix  $\mathbf{Q}$  is factorized to smaller matrices of  $\mathbf{W}$  and  $\mathbf{H}$ , where  $\mathbf{W}$  is  $k \times r$  matrix,  $\mathbf{H}$  is  $r \times m$  matrix where  $r$  corresponds to number of basis vectors. Hence, matrix  $\mathbf{WH}$  would has the same size and value as the matrix  $\mathbf{Q}$ . Their relationship is determined by:

$$\mathbf{Q} = \mathbf{WH} \quad (2)$$

The number of odor components to be approximated is equal to the number of basis vectors  $r$ . Here, the shape of each odor component corresponds to each basis vector in the row of the factorized matrix  $\mathbf{H}$ .

Technically, NMF-IS can only be utilized in odorant analysis in numerical form. However, proving the odorant analysis in the physical form was necessary to reinforce the result. Here, the nonnegative least squares method (NLSM) was utilized to calculate the recipe to create odor components as well as the recipe for odor approximation in the physical form [15]. The NLSM with Itakura-Saito divergence was utilized in this study. The divergence utilized in NMF and NLSM should be consistent to achieve a higher approximation accuracy. For easier understanding, the illustration of odorant analysis is shown in Fig S1 [15].

### D. SENSORY EVALUATION

In this study, we prepared two sets of physically approximated odors based on odorant analysis with original essential oils and extracted essential oils. NLSM were utilized twice for adjustment of the recipe for odor approximation. Recipe adjustment was necessary to transform odor approximation from numerical results to physical ones. First, we approximate odor components by mixing essential oils based on the recipe from the first NLSM result. Then, we approximated the target odor by mixing odor components based on the recipe from the second NLSM result. Seven different samples from seven primary groups of essential oils based on the book by Wanda Sellar [24] were chosen as the target for odor approximation and utilized for the sensory test.

Duo-trio sensory test was performed for the evaluation of approximated odor [25], [26]. The aim of the sensory test was to evaluate whether the smell similarity to reference odor is different between approximated odor from original essential oils and extracted essential oils prior to suppression of fixatives in extracted essential oils. Even though extracted essential oils originally contained fixatives before the suppression of interference, fixatives were not included in the approximated odor. We already confirmed that fixatives were indeed odorless in our previous study [18]. Thus, fixatives were omitted in the approximated odors.

The procedure of the duo-trio test is shown in Fig. 3. In the duo-trio sensory test, a subject was first exposed to the reference odors (original target essential oils). Then, the subject needed to sniff two approximated odors (one based on original essential oils and another one based on extracted essential oils). Later, the subject needed to choose one of the approximated odors closer to the reference odor. Each subject conducted a duo-trio test for seven different sets of reference and approximated odors. The odor was provided to the subject in a vial. The subject needed to sniff the odor from the vial.

Evaluation of sensory test result was conducted by calculating  $\alpha$ -risk with a 5% threshold. We also calculated the  $z$ -score for further evaluation [25]. The  $z$ -score equation for the sensory test is as follows:

$$z = (a - 0.5b) / \sqrt{0.25b} \quad (3)$$

where  $a$  is the number of the answer from the subject and  $b$  is the number of the tests.

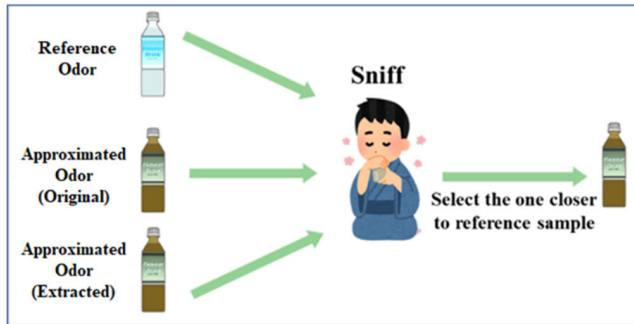


FIGURE 3. Illustration of the duo-trio sensory test.

### III. RESULTS

#### A. INTERFERENCE SUPPRESSION RESULT

One hundred eighty mass spectrum data of physical mixed samples were measured. First, we applied preprocessing to eliminate small noises that might appear during measurement. The coefficient-of-variation based preprocessing was performed in this study. First, we searched for coefficient-of-variation at the highest intensity within all measured  $m/z$  regions of an averaged mixed sample data. If the coefficient-of-variation of each  $m/z$  was higher than the coefficient-of-variation at the highest  $m/z + 1\%$ , then we regarded it as noise, and its value was replaced with zero [15].

In our previous study, we proved that essential oils that being used as samples in our inventory were indeed pure [18]. However, the study was only conducted in the numerical phase. Hence, reinforcing the study with physical samples became essential. Here we applied ICA to the physically measured mixed sample. First, we intentionally added the fixative mass spectrum to mixed sample data and applied ICA to the data. Thereafter, we extracted six independent components (ICs) that correspond to either fixatives or pure odorant samples. First, we identified ICs that corresponded to fixatives. Then, the remaining IC was considered as pure odor samples without the influence of fixatives. Finally, we extracted pure odor from 180 mixed samples. The extracted pure odor is called extracted essential oils.

After we extracted the pure odor, we compared the correlation coefficient between ICs and their corresponding odor samples from artificially generated data from our previous study [18] and physically measured data in this study which is shown in Fig 4. The average correlation coefficient was 0.874 for artificially generated data and 0.888 for physically measured data. The comparison result indicates that the ICA result from artificially generated data and physically measured data were close. Hence, we could proceed to odorant analysis for further evaluation.

#### B. ODORANT ANALYSIS RESULT

First, odorant analysis was performed by calculating NMF-IS. We calculated NMF-IS to large-scale mass spectrum data of original essential oils as well as extracted essential oils with the different number of basis vectors. Then, we applied the

nonnegative least squares method (NLSM). The evaluation of odorant analysis in the numerical form was done by calculating Itakura-Saito divergence as reconstruction error. The reconstruction error can be calculated based on the result of the second NLSM. The comparison of NLSM from original essential oils and extracted essential oils with different number of basis vectors is shown in Figs 5a and b. We calculated IS divergence between the mass spectrum of target odor and approximated odor as reconstruction error.

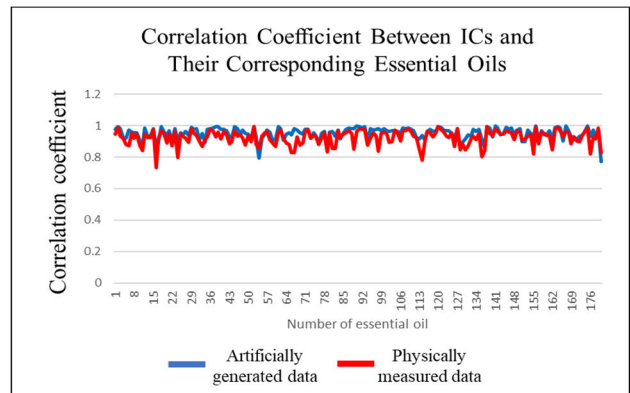
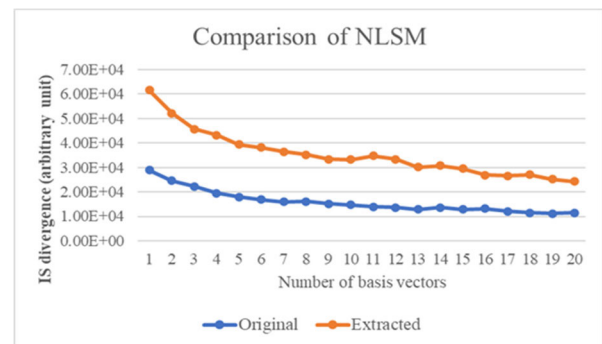
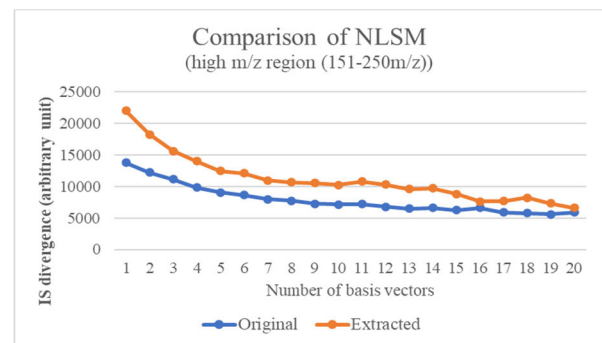


FIGURE 4. The correlation coefficient between ICs and their corresponding essential oils for artificially generated data and physically measured data.



(a)



(b)

FIGURE 5. Comparison of reconstruction error of second NLSM result for (a) full 50 to 250  $m/z$  region and (b) high 151 to 250  $m/z$  region.

The result of NLSM shows that NLSM with original essential oils has lower reconstruction error than NLSM

with extracted essential oils in regards of the full 50 to 250  $m/z$  region. However, in the high 151 to 250  $m/z$  region, both NLSM results became closer. In our previous study, we revealed that lower reconstruction error at a high 151 to 250  $m/z$  region tends to contribute to higher approximation accuracy in terms of the sensory test. This was due to the human nose's tendency to be more sensitive to low volatile compounds in the high  $m/z$  region [15]. Since the difference between NLSM with original essential oils and extracted essential oils became very small in the high  $m/z$  region, we can say that they have a close approximation result close to each other.

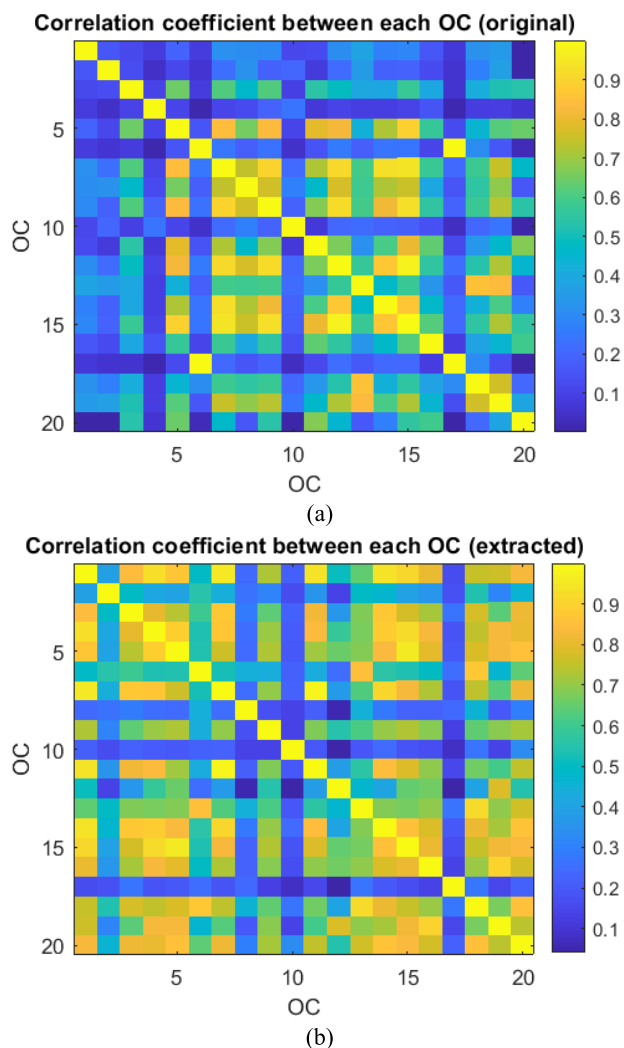
As mentioned before, odor reproduction needs a set of odor components for odor approximation. Our previous study revealed that 20 odor components were feasible for odor reproduction [27]. Therefore, we proceeded with odor components of 20 for physical odorant analysis in this study.

Subsequently, to choose the candidate for the number of odor components, we calculated the Pearson correlation coefficient between each odor component (OC) for 20 odor components. This was performed to evaluate the redundancy of each odor component. The result of the Pearson Correlation coefficient is shown in Figs 6a and b. The results show that the average correlation coefficient between each odor component was 0.4115 for original essential oils and 0.572 for extracted essential oils.

After the first analysis was performed, we approximated the odor components in the physical form by blending essential oils based on the first NLSM result. Two sets of odor components were made in this study. A set of odor components based on original essential oils and another set of odor components based on extracted essential oils. Since we already confirmed that fixatives were indeed odorless [17], [18] and we tried to prevent odors from lingering during sensory evaluation with the human subject, thus, we omitted the fixatives in making odor components based on extracted essential oils.

We measured the physically approximated odor components with MS and calculated the Pearson correlation coefficient between the mass spectrum of physically approximated odor components and numerically calculated odor components. This was done to evaluate whether the physical approximation was successful or not. The correlation coefficient between target odors and approximated odors were 0.9722 for original essential oils and 0.9942 for extracted essential oils. The correlation coefficient was very high, meaning the physically approximated odor components were done successfully.

The next step after approximated odor components was physically approximated target odor, or what we called odor reproduction of target odor. Seven different target odors were approximated by blending odor components using blending recipe earned from the second NLSM result. We approximated two sets of target odors. The first one was based on odor components from original essential oils, and another one was based on extracted essential oils. After odor approximation was done, we measured the mass spectrum of



**FIGURE 6.** Result of Pearson correlation coefficient between each odor component (OC) of (a) odor components based on original essential oils (0.4115) and (b) odor components based on extracted essential oils (0.572).

approximated odors with MS. Then we calculated Pearson Correlation Coefficient between target and approximated odors. The information on seven approximated odors and the result of the correlation coefficient between target and approximated odor is shown in Table 2.

The correlation coefficient was calculated for the evaluation of the accuracy of odor approximation. A higher value of the correlation coefficient means higher approximation accuracy. Moreover, examples of the mass spectra comparison of clove bud reference odor and the approximated odor based on original and extracted essential oils are shown in Figs 7a and b. Based on these results, we can say that approximation of the target odor was successfully performed even if there were slight differences in the correlation coefficient between the target odor and the approximated odors.

Besides evaluating physically approximated odors, we also calculate the correlation coefficient between target odor

and approximated odor in the numerical calculation for all 180 essential oils. The average correlation coefficient between target odor and approximated odor for all 180 essential oils was 0.8952 for original essential oils and 0.8322 for extracted essential oils. Although there was a slight difference in the correlation coefficient based on original essential oils and extracted essential oils, the difference is negligible. Hence, the correlation coefficient analysis reveals that both have similar odor approximation accuracies.

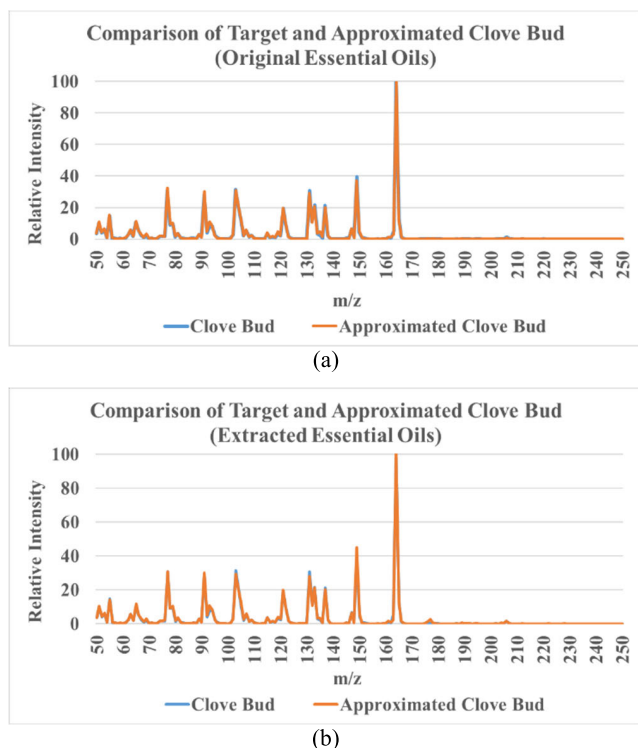


FIGURE 7. Comparison of clove bud reference odor and approximated odor using odor components based on (a) original essential oils and (b) extracted essential oils.

TABLE 2. Approximated odors and result of correlation coefficient.

Samples	Correlation coefficient		Essential oil group
	Original	Extracted	
Clove bud	0.9784	0.998	Spices
Elemi	0.9648	0.8938	Resins
Orange Sweet	0.9658	0.9596	Citrus
Sandalwood	0.866	0.7912	Exotics
Chamomile (Maroc)	0.9574	0.8876	Flowers
Cajeput	0.9709	0.9972	Trees
Mentha piperita mitcham	0.9778	0.9315	Herbs

C. SENSORY EVALUATION RESULT

Even though the suppression of interference was successful in numerical analysis, sensory evaluation with a human nose

would be indispensable. Thus, a sensory test to evaluate the similarity of smell was needed to prove the result of interference suppression. Therefore, the duo-trio test was performed with seven different reference samples (clove bud, elemi, orange sweet, sandalwood, chamomile maroc, cajeput, mentha pipetha mitchan). The participants for the sensory test were 22 nonexperts aged between 17 and 56 years old (females and males). The subject sniffed a vial containing the reference sample firsthand before sniffing other vials containing approximated odors that needed to be compared and chose the one closer to the reference smell. The sensory evaluation was conducted in the room with sufficient airflow to prevent the smell from remaining in the air. Such a condition was essential to prevent misjudgment during sensory evaluation.

The result of the duo-trio sensory evaluation with the respective set is shown in Table 3. The  $\alpha$ -risk with a 5% threshold needed 16 or more participants to choose either one to be considered as there is a significant difference between tested samples in the duo trio test [25]. We also calculated each sample’s z score ( $p < 0.05$ ). The z score must be below 1.717 to prove that there is no significant difference between tested samples in the duo trio test. The result of the sensory evaluation showed that all seven samples had a number of participants choosing either side less than 16 and a z score lower than 1.717. Hence, it can be concluded that there was no significant difference of similarity to target essential oil between approximated odor based on original essential oil and one based on extracted essential oil.

Approximated odor based on original odor is not exactly same as approximated odor based on extracted one, but roughly similar. Thus, this sensory evaluation result reinforced the result of our method in the suppression of interferences where suppression of odorless fixatives does not affect the smell characteristic of extracted sample.

TABLE 3. Duo-trio sensory test result.

Reference odor (target essential oil)	Subject response		z score
	Approximated by original essential oil	Approximated by extracted essential oil	
Clove bud	14	8	1.279
Elemi	8	14	1.279
Orange sweet	12	10	0.426
Sandalwood	9	13	0.853
Chamomile (Maroc)	10	12	0.426
Cajeput	7	15	1.706
Mentha piperita mitcham	9	13	0.853
Total test number	69	85	1.289

IV. DISCUSSION

Although mass spectrometry has the advantages of measuring odorant samples with high sensitivity, reproducibility, and fluidity, it still has limitations [6], [12]. For example, MS can

detect chemical components remaining undetected in terms of the human sense of smell. This might cause problems when the odorant analysis was performed with MS. Moreover, MS cannot eliminate noise or interference on its own [12], [15]. Hence, additional methods to be applied to MS data to eliminate those problems are necessary.

In our previous study, we evaluated the suppression of interference only in the numerical solution [18]. Since we needed to expand our research in odorant analysis with an increasing number of odor samples, proving our method in the physical samples became necessary. We needed to eliminate the unwanted chemical compound in the odorant samples that cause interference or noise before odorant analysis. Hence, we developed this suppression of interference to open a pathway for smoother odorant analysis without unwanted chemical compounds. Previously, our study was limited only to numerical analysis. However, we could expand the study by utilizing physically measured samples. Then, successfully performed the odorant analysis in the physical samples in this study.

Despite the influence of fixatives in the physical mixed samples, ICA could extract pure essential oils that have a close odorant characteristic with original essential oils during odorant analysis with humans as the subject. Duo-trio has general application with test subject of at least 15 [25]. Even though human has subjectivity, the increase in the number of subjects as well as introduction of the statistical test reduced the subjectivity. This study reinforced the possibility of interference suppression in the odorant sample utilizing mass spectrum data with a feasible result.

Besides qualitative odorant analysis during sensory test, odorant analysis utilizing mass spectrometry can provide quantitative odorant analysis result. Mass spectrometry measurement can provide quantitative data of odorant by intensity at each mass-to-charge ratio ( $m/z$ ) where each odorant has its own characteristic.

Furthermore, the procedures applied in this study (ICA and NMF) might be not sufficient to reproduce in strictly numerical manner. However, we could reproduce the ICA model with different set of samples (perfume) and fixatives with the same parameters with similar result [28]. Change of parameters such as iteration and cost function are needed to get the good result. Moreover, parameter of the ICA and NMF used in this study is provided in Table S3.

Widely available odorant samples, such as perfumes, mostly contain fixatives in it [17], [29]. In our recent study, we gathered perfume as many as possible and measured the mass spectrometry data. Then we applied ICA to the perfume MS data. Even though the numerical result could be utilized to extract pure perfume data, a preliminary study was needed to confirm the performance of ICA in the physical samples with known pure samples a priori. Nevertheless, this study proved the suppression of interference with ICA can be done. Hence in the near future, we can add pure perfume data as well as more odorant samples to the dataset for odorant analysis.

Eventually, our proposed method can omit the necessity of specifically designed odorant samples for odorant analysis since the suppression of interference can be done numerically. Nevertheless, further studies with different kinds of odorant samples for odorant analysis are still needed.

## V. CONCLUSION

In this study, our method of suppressing interference of unwanted compounds in odorant samples utilizing ICA was successfully applied not only in the numerical result but also in the physical samples. Odorant analysis in mass spectrum space and sensory evaluation conducted using physical samples in this study reinforced the result of our previous numerical result [18]. Hence, we can explore a much wider selection of odorant samples for odorant analysis without fear of interferences caused by unwanted chemical compounds.

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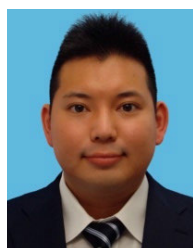


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