Measuring Odor Threshold Using a Simplified Olfactory Measurement Method

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Abstract

This study used the Simplified Olfactory Measurement Method to clarify the fundamental characteristics of substances with offensive odors present in human living environments and to measure the odor detection threshold of these substances, which have relatively high boiling points and high molecular weights. Results showed that propanoic acid was the highest in those substances, with an odor threshold of 49 ppb, and phenylacetic acid was the lowest in those substances, with an odor threshold of $2.4 \times 10^{-6}$ ppb. Although there was a gap between the Simplified Olfactory Measurement Method and the Triangle Odor Bag Method, the former still fell in the range of thresholds that can occur between panels. Furthermore, the threshold for the relationship between thresholds of straight-chain fatty acids and molecular carbon numbers gradually decreased for carbon numbers from three to five and increased again in six. That trend resembles the result of the Triangle Odor Bag Method.

Keywords: Simplified Olfactory Measurement Method, odor concentration, odor threshold, degree of certainty, Triangle Odor Bag Method

1. Introduction

We studied unpleasant odors in the living environment and clarified the distinctive characteristics of two odors: phenylacetic acid, which is a component in the odor of urine, and nonanoic acid, which is a component in the odor of an oily body (Daikoku et al. 2018). We analyzed odors that adhered to the surface of products in the human living environment using gas chromatography-olfactometry-mass spectroscopy (sniffing GC-MS) and discovered many substances with offensive odors (Uchiyama et al. 2016). It is generally considered that the transpiration of these substances into the atmosphere is difficult because they have relatively higher boiling points and molecular weights than the offensive odor substances regulated by Offensive Odor Control Law in Japan (Japan Association of Odor Environment 2012). The substances studied were not recognized as offensive odor substances to date as their fundamental characteristics as odor substance had not been elucidated.
The odor threshold is the minimum concentration at which the presence of an odor can be detected, and it is an important indicator in the quantification of olfactory perceptions. It is used in the wide field related to odors to correlate equipment-based analysis with odor sensory evaluation or to measure odors.

The Triangle Odor Bag Method (Iwasaki et al. 1978) (hereafter “the official method”) was set by the Ministry of the Environment in Japan as a standard odor measurement method, and a large number of odor thresholds have been measured so far (Nagata and Takeuchi 1990). On the other hand, the Simplified Olfactory Measurement Method (hereafter “the simplified method”) was developed recently as different technique based on the official method, improves it (Hatano 2010). The simplified method enables the measurement of an odor in a short time and with a small number of people in the field.

The purpose of this study was to measure the odor threshold of the offensive odor substances that we discovered recently, using the simplified method, and to elucidate their fundamental characteristics. Furthermore, we measure the same substances using both the simplified and the official methods to examine the differences that occur based on the methodology used.

2. Methods
2-1. Odor sample and preprocessing
The name, formula, CAS number, boiling point, and molecular weight of the 19 odor substances that we measured in this study are shown in Table 1. A sampling bag (Flex-sampler, 10 L, Omi Odor-Air Service Corporation) was filled with 5 L of nitrogen. The quantity of each reagent, which we had calculated beforehand, was measured with a microsyringe and injected into the sampling bag from its rubber cap. Then the rubber cap was wound with sealing tape and the sampling bag was kept in a thermostatic chamber at 100 °C for one hour to let the reagent be completely vaporized by the heat. Afterwards, it was kept to stabilize at 25°C for two hours and used in the experiment. If the reagent was in a liquid state at room temperature, it was used for the experiment as was; however, if it was in a solid state due to its high boiling point, it had to be pretreated to be measured with the microsyringe. Among the reagents, decanoic acid and undecanoic acid melted in a water bath, which we confirmed by viewing that they turned into liquids. Other solid reagents weighed 0.7 gram and were put in a vial with ethanol, brought to a total of 7 gram, and adjusted to 10 wt%. The vials were put into a 100 °C water bath, and it was visually confirmed that every reagent turned back into a liquid before they were brought back to normal temperature and used in the experiment. The odor thresholds of 19 reagents were measured using the simplified method, and 6 of those were also measured using the official method.

2-2. Measurement using the official method
The odor concentration was measured using the Triangle Odor Bag Method that follows the outlet sample method as per 63rd notification of the Agency of the Environment, Japan, from 1995 (latest revision: 79th notification of the Ministry of the Environment, 2016). Odorless air was filled in three bags (Flek-sampler, 3 L, Omi Odor Air Service Corporation) and

| Name                   | Formula   | CAS No. | Boiling point | Molecular weight |
|------------------------|-----------|---------|---------------|------------------|
| 1 Acetic acid          | C₂H₄O₂    | 64-19-7 | 118           | 60.05            |
| 2 Propanoic acid       | C₃H₆O₂    | 79-09-4 | 141           | 74.08            |
| 3 Butanoic acid        | C₄H₈O₂    | 107-92-6| 163           | 88.11            |
| 4 Pentanoic acid       | C₅H₁₀O₂   | 109-52-4| 186           | 102.13           |
| 5 Heptanoic acid       | C₆H₁₄O₂   | 111-14-8| 223           | 130.18           |
| 6 Octanoic acid        | C₇H₁₄O₂   | 124-07-2| 238           | 144.21           |
| 7 Nonanoic acid        | C₈H₁₆O₂   | 112-05-0| 270           | 158.24           |
| 8 Decanoic acid        | C₉H₁₈O₂   | 334-48-5| 268-270       | 172.26           |
| 9 Undecanoic acid      | C₁₀H₂₀O₂  | 112-37-8| 284           | 186.29           |
| 10 Dodecanoic acid     | C₁₁H₂₂O₂  | 143-07-7| 225           | 200.32           |
| 11 Tetradecanoic acid  | C₁₂H₂₄O₂  | 544-63-8| 250           | 228.37           |
| 12 Hexadecanoic acid   | C₁₃H₂₆O₂  | 57-10-3 | 390           | 256.42           |
| 13 Octadecanoic acid   | C₁₄H₂₈O₂  | 57-11-4 | 386           | 284.48           |
| 14 4-methyl hexanoic acid | C₁₅H₁₈O₂ | 1561-11-1| 109-112      | 130.18           |
| 15 3-methyl pentanoic acid | C₁₅H₂₀O₂ | 105-43-1| 196-198       | 116.16           |
| 16 Octanolactone       | C₁₆H₁₈O₂  | 104-50-7| 234           | 142.19           |
| 17 Benzothiazole       | C₁₇H₁₄NS  | 95-16-9 | 235           | 135.19           |
| 18 Benzic acid         | C₁₈H₁₂O₂  | 65-85-0 | 249           | 122.12           |
| 19 Phenylacetic acid   | C₁₉H₂₈O₂  | 103-82-2| 266           | 136.15           |
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the primary odor sample prepared as described in section 2-1 was injected into one of those to act as the initial examination concentration. The panels of testers compared the samples by smelling them and answered the odor-filled bag number. If the test of initial concentration was correct answer, the next test diluted by three orders of magnitude (3, 10, 30, 100) and this test was repeated until an incorrect answer was given. The individual odor thresholds were the logarithmic means of a multiple of the order of magnitude of the dilution of an incorrect answer and the lowest correct answer. The odor threshold was found from the mean of the individual odor thresholds for the six panels.

2-3. Measurement using the simplified method

A flowchart of the simplified method is shown in Figure 1 and the answer sheet is shown in Figure 2 (Ministry of the Environment in Japan 2018a). Odorless air was filled into two odor bags and the primary odor sample prepared as described in section 2-1 was

![Flowchart of odor concentration measurement using the simplified method](image)

Figure 1 Flowchart of odor concentration measurement using the simplified method

![Answer sheet for the simplified method used in this study](image)

Figure 2 Answer sheet for the simplified method used in this study
injected into one of those to act as the first examination concentration. The panels compared the two bags containing the odorous gas and the odorless bag and one clearly odorless bag by smelling them, and answered the correct odorous bag number, the odor intensity and degree of certainty. If the test of first concentration produced the correct answer, the next test diluted the concentration of the odor three times and repeated this operation until the answer was incorrect. Even if the answer about the odorous bag number was correct, when the odor intensity and degree of certainty were both low, we evaluated the same test conditions again. The individual odor threshold was the logarithmic mean of a dilution multiple of incorrect answer and the maximum of the correct answer; however, if the evaluation panels answered correctly twice consecutively when the odor intensity was “0” and degree of certainty was “a”, the individual odor threshold was the logarithm of that dilution factor. The odor threshold was found based on the mean of the individual odor thresholds from all the panels.

Table 2 compares and summarizes the official method and the simplified method (Ministry of the Environment in Japan 2018a). The simplified method reduced the scale of the examination, since there were merely three people on the panel and the samples were small. Dedicated examination operators were necessary to prepare the samples in the official method, but in the simplified method the people on the panels conduct the examination themselves. The number of accidental correct answers was reduced because both the odor intensity and the degree of certainty about the reliability of the examination were recorded, as well as the number of the odor-filled bag.

2-4. Quantitative analysis of gas concentration

The sampling bag with primary odor sample was connected to a sampling tube with silicone tube, and the primary odor sample was pumped from the sampling pipe at a flow rate of 500 mL/min using an air pump (MP-Σ300NII, Shibata Scientific Technology Ltd.,) that was concentrated to sampling tube. We took care so that the area of the silicone tube exposed to the gas sample was small, so that the gas was not absorbed inside the silicone tube. The sampling tube was quantified using GC-MS under the conditions shown in Table 3. The quantity of the substance contained in the sample gas was converted into mols to calculate the gas concentration.

2-5. Calculating odor threshold

The odor index \((OI)\) was obtained from the arithmetical mean of the average of the individual odor thresholds \((T)\) found using the simplified and the official methods (equation 1), and converted into odor concentration \((OC)\) (equation 2). The gas concentration \((C)\) of the primary odor found according to the methods in section 2-4 was divided by \(OC\) to give odor threshold of

| Method | Official | Simplified |
|--------|---------|------------|
| Number of people on the Panel | Six or more | Three* |
| Number of samples to compare | 3 bags; 1 odor bag and 2 odorless bags | 2 bags; 1 odor bag and 1 odorless bag** |
| Number of the bags per test | 72 bags | 39 bags |
| Measurement time | 20-40 min | 10-20 min |
| Evaluation items | • the number of the correct bag | • the number of the correct bag | • odor intensity | • degree of certainty of the answer |

* One person serves concurrently as a test operator.
** One clearly odorless bag is prepared for comparison.

Table 3 Apparatus and parameters used in the gas chromatography-mass spectrometry analysis

| Apparatus/Parameter | Details |
|---------------------|---------|
| Gas chromatograph   | GC 7890B, Agilent Technologies |
| Mass spectrometer   | MS 5975C, Agilent Technologies |
| Sample preparation equipment | MultiPurpose Sampler MPS, TDU, Gerstel KK |
| Sampling tube       | Tenax TA tube, 013741-000-KK, Gerstel KK |
| Temperature of the heated sampling tube | 40℃-(720℃/min)-300℃ |
| Cryofocus           | 10℃ |
| Injection method    | Splitless |
| Capillary column    | HP-INNOWAX 19091N-133 30 m / 0.25 mm / 0.25 μm |
| Oven                | 60℃-(10℃/min)-250℃ |
| SIM monitor ion     | propanoic acid: m/z = 74, octanolactone: m/z = 85, benzothiazole: m/z = 135, benzoic acid: m/z = 105, phenylacetic acid: m/z = 136, others: m/z = 60 |
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2-6. Date of measurement
The measurement using the simplified method was carried out from September 15, 2016 to December 15, 2016, and those using the official method were carried out from June 22, 2017 to September 12, 2017.

2-7. Test site
The test site was a quiet, unscented meeting room kept at 25 ℃, at a relative humidity of 50 %. There was at least one seat between the panelists. The panels were prepared in another room.

2-8. Panels
The panels were healthy male and female university students, 19-22 years old, who passed a screening test based on test 5-2 of the T&T olfactometer method (Iwasaki et al. 1983a). Six out of seven people were chosen as the panel for the official method, and three out of eight people were chosen for the simplified method. Several odor substances measured beforehand confirmed that there was no remarkable difference of olfactory perception between the panels, because the substances evaluated by the panels were different. One of three panels participated in the evaluation and prepared the samples using the simplified method. All experiments with humans were conducted according to the Declaration of Helsinki.

3. Results
The OC and C obtained from the simplified and official methods, and the OT obtained from diving C by OC are summarized in Table 4. The odor threshold of No. 1 to No. 4 were referred to Nagata and Takeuchi (1990), and No.7 and No.19 were referred to Daikoku et al. (2016).

The highest odor threshold among the odor substances was 49 ppb of propanoic acid, and the lowest was 2.4×10⁻⁶ ppb of phenylacetic acid. Ethanol, which we used to dissolve the solid reagent, did not disturb this evaluation because its odor threshold was 5.2×10⁻⁴ ppm (Nagata and Takeuchi 1990), which is higher than those of the target substances.

When compared, the OT of the simplified method was 0.340 and 0.094 times smaller than that of official method for butanoic acid and undecanoic acid, respectively. The OT of the simplified method was 2.9, 5.4, 38, and 22 times larger than that in the official method for octanoic acid, nonanoic acid, 3-methylpentanoic acid, and benzoic acid, respectively.

However, there was difference between the simplified method and the official method based on the

| Substance name          | Simplified method (This study) | Official method (This study) | Past studies |
|-------------------------|--------------------------------|-----------------------------|--------------|
|                         | OC                  | C                         | OT          | OC                  | C                         | OT          |
| 1. Acetic acid          | 4.8×10⁻⁵           | 6.6×10⁻¹                   | 1.4×10⁴     | -                   | -                         | -           | 6.0×10⁻⁷   |
| 2. Propanoic acid       | 9.6×10⁻⁴           | 4.7×10⁻³                   | 4.9×10²     | -                   | -                         | -           | 5.7×10⁻⁷   |
| 3. Butanoic acid        | 4.0×10⁻²           | 1.7×10⁻²                   | 4.4×10⁵     | 2.5×10⁻²           | 3.1×10⁵                   | 1.3×10⁵     | 1.9×10⁻⁷   |
| 4. Pentanoic acid       | 1.2×10⁻⁶           | 1.2×10⁻¹                   | 1.0×1⁰      | -                   | -                         | -           | 3.7×10⁻⁴   |
| 5. Heptanoic acid       | 4.8×10⁻⁵           | 2.9×10⁻³                   | 6.0×1⁰      | -                   | -                         | -           |              |
| 6. Octanoic acid        | 7.9×10⁻⁶           | 2.7×10⁻⁶                   | 3.4×1⁰      | 8.0×10⁻¹           | 9.6×10⁻¹                   | 1.2×10⁰     | -           |
| 7. Nonanoic acid        | 5.0×10⁻¹           | 3.3×10⁻ⁱ                   | 6.5×1⁰      | 4.0×10⁻²           | 4.9×10⁻¹                   | 1.2×10⁻¹     | 4.5×1⁰     |
| 8. Decanoic acid        | 2.0×10⁻²           | 6.1×10⁻²                   | 3.0×1⁰      | -                   | -                         | -           |              |
| 9. Undecanoic acid      | 7.9×10⁻¹           | 1.2×1⁰                    | 1.6×1⁰      | 1.0×1⁰²           | 1.7×1⁰⁰                   | 1.7×1⁰²     | -           |
| 10. Dodecanoic acid     | 5.0×10⁻²           | 2.1×1⁰                    | 4.2×1⁰³     | -                   | -                         | -           |              |
| 11. Tetradecanoic acid  | 5.0×10⁻¹           | 3.9×1⁰                    | 7.8×1⁰³     | -                   | -                         | -           |              |
| 12. Hexadecanoic acid   | 2.5×10⁻²           | 1.4×1⁰                    | 5.5×1⁰⁶     | -                   | -                         | -           |              |
| 13. Octadecanoic acid   | 1.6×1⁰¹            | 1.7×1⁰                    | 1.1×1⁰⁴     | -                   | -                         | -           |              |
| 14. 4-methyl hexanoic acid | 4.8×10⁻⁴       | 3.4×1⁰⁻¹                   | 7.0×1⁰⁴     | -                   | -                         | -           |              |
| 15. 3-methyl pentanoic acid | 1.2×10⁻⁶       | 1.2×1⁰⁻²                   | 9.6×1⁰³     | 5.5×1⁰⁴           | 1.4×1⁰³                   | 2.5×1⁰⁶     | -           |
| 16. Octanalactone       | 7.5×10⁻⁶           | 1.5×1⁰⁻⁵                   | 2.0×1⁰⁴     | -                   | -                         | -           |              |
| 17. Benzothiazole       | 1.5×10⁻⁶           | 5.2×1⁰⁻⁵                   | 3.5×1⁰⁵     | -                   | -                         | -           |              |
| 18. Benzoic acid        | 1.3×10⁻²           | 1.0×1⁰⁻⁶                   | 7.8×1⁰⁵     | 4.0×1⁰⁻²           | 1.4×1⁰⁻¹                   | 3.6×1⁰⁵     | -           |
| 19. Phenylacetic acid   | 1.5×10⁻⁵           | 3.6×1⁰⁻⁴                   | 2.4×1⁰⁸     | -                   | -                         | -           | 6.8×1⁰⁴     |
substances. Thresholds fluctuated within a panel, and were known to fluctuate by 100 to 1,000 times between panels (Iwasaki et al. 1983b) who passed the selection test (Saito et al. 2014). The difference was within these limits. It was considered that the simplified method would be easily affected by individuals’ sniffing ability or technique, the palatability of the odors given the fewer people on the panels (compared to the official method), or because the maximum and the minimum individual odor thresholds were not removed, but their influences were small in these measurements. We wanted to separately investigate the factors, for instance age, gender and quantitative determination error of GC-MS, that influence the accuracy of the measurement result.

4. Discussion

4-1. Examination of the panel attribution of the official method

To grasp the distribution of the ability to detect odors, the odor threshold measured according to the official method, using the T&T olfactometer method of seven panels, is shown in Table 5. Three odors that resembled each other in kind of odor and molecular structure were chosen from five standards odors. Thus we considered that the panel adopted this time was appropriate because no panel member had results that were extremely far from the average.

| Standard odors  | Logarithm of odor thresholds |
|-----------------|-----------------------------|
|                 | -4.0 | -4.5 | -5.0 | -5.5 | -6.0 | -6.5 | -7.0 | Avg.   |
| Phenylethylalcohol | 1    | 0    | 3    | 2    | 0    | 1    | 0    | -4.7  |
| i-valeric acid   | 0    | 0    | 1    | 1    | 2    | 2    | 1    | -5.6  |
| γ-undecalactone  | 0    | 2    | 3    | 2    | 0    | 0    | 0    | -4.8  |

4-2. The relationship between boiling point, molecular weight, and odor threshold

Figure 3 shows the relationship between molecular weight and odor threshold as measured using the simplified method and Figure 4 shows the relationship between boiling point and odor threshold. The vertical axis is a logarithm of the odor threshold.

The odor threshold decreased as the molecular weight increased, and a similar tendency was seen in previous studies using the official method by (Nagata and Takeuchi 1990) and Saito et al. (1985) The correlation coefficient \( r = 0.283 \) in the official method (Ministry of the Environment in Japan 2018b), and \( r = 0.184 \) in the simplified method in this time, both of them was low regardless of the measurement method. In addition, the odor threshold became small, as the boiling point became high, too. The correlation coefficient was small, and the relationship between the odor threshold, molecular weight, and boiling point was not significant. Therefore, it is considered that the odor threshold cannot be determined only from molecular weight or boiling point, and the quality of odor or the molecular structure of the odor substances must be included, and we did not examine in this time.

**4-3. Examination of fatty acids**

The relationship between the carbon number and odor threshold of the straight-chain saturated fatty acids substances.
that are a part of this measurement are shown in Figure 5. The odor threshold of the simplified method is a black circle, the official method used in the previous study by Nagata and Takeuchi (1990) is a white triangle, the odor threshold in water is a white square (Fazzalari 1978), and each threshold is shown on a logarithmic scale.

The Odor thresholds were highest when the carbon number was three, gradually decreased, and then increased again when the carbon number was six. This is similar to the shifts seen in the previous study by Nagata and Takeuchi (1990), which used the official method. After a carbon number of six, the odor threshold increased and reached its highest when the carbon number was nine, and the trace gradually decreased. Even if the odor substances had the same straight-chain saturated fatty acid functional groups, it was difficult to predict and judge odor threshold value using only the carbon number.

There was a broader variation in the odor threshold in air based on the official method and the simplified method compared to that in water. The odor threshold in air measured the sample that had been adjusted by heating the reagent forcibly and vaporized; however, the odor threshold in water evaluated the odor that had been volatilized from the water solution (Guadagni and Buttery 1978).

For this reason, it is thought that is possibly caused by the difference between odor substances are hard to be provided. It is necessary to examine another factor such as the saturated vapor pressure.

5. Conclusion

We measured odor concentration of odor substances in the living environment using a simplified olfaction method, quantified the gas concentrations of primary odors using GC-MS, and calculated their odor thresholds.

The results clarified the following points.

(1) Propanoic acid was the most common substance and its odor threshold was 49 ppb; phenylacetic acid was the least common substance and its odor threshold was 2.4×10^-6 ppb. Although there was difference between the simplified and the official methods depending on the substances tested, the thresholds are within the individual fluctuations. The differences in the odor threshold due to differences in methodology were small.

(2) Although the odor threshold decreased as the molecular weight and boiling point increased overall, there was no significant correlation between them.

(3) The relationship between the carbon number and the odor threshold of straight-chain saturated fatty acids increased up to a carbon number of three, decreased up to a carbon number of five, and increased again up to a carbon number of six. This trend resembles that seen with the official method of previous study.

We would like to continue to measure other odor substances with the simplified method in future.

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