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Preventive effect of flavor/fragrance components on SARS-CoV-2 infections

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ABSTRACT

The SARS-CoV-2 infection has spread to various areas of the world, and the number of infected people, seriously ill people, and deaths have increased in 2020~2023. It is important to suppress the spread of virus from infected people to non-infected people in order to prevent the disease from becoming more severe. To protect widespread of virus, flavor/fragrances composition was selected as a convenient effective material to protect the inhibition. It was previously investigated whether flavor/fragrances composition inhibit the binding between the receptor-binding domain (RBD) of the SARS-CoV-2 spike protein and host angiotensin converting enzyme 2 (ACE2) in the infection model assay. This binding lead to natural infection of SARS-CoV-2 to tissues. In a previous report, it was found that some Flavor/fragrances compositions strongly inhibited the binding between RBD and ACE2. To clarify whether these flavor/fragrances compositions actually inhibit the infection of SARS-CoV-2, the inhibition assay of infection to VeroE6/TMPRSS2 cells, the inhibition model in vitro, were performed by the treatment of these compositions. Some flavor/fragrances compositions excepting for cinnamyl alcohol, 0.25 %, strongly inhibited the infection of SARS-CoV-2 to VeroE6/TMPRSS2 cells because cinnamyl alcohol could not be completely melted by PBS (pH 7.4) containing 1.5 % Tween 20 and 0.5 % BSA. Among fragrance compounds, cinnamon flavor and cinnamon mint had stronger inhibition effects on the infection effects on SARS-C0V-2 than others. The strategy of using flavor/fragrances compositions such as cinnamon flavor and cinnamon mint may be useful to protect widespread of SARS-CoV-2 in their daily lives.

1. Introduction

The coronavirus infection (COVID-19) has spread to various areas of the world, and the number of infected people, seriously ill people, and deaths have increased in 2020~2023 (Davis et al., 2023; Hu et al., 2021). It is less pathogenic than previous coronavirus outbreaks like Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS), and many patients recover even if they develop symptoms (Li et al., 2021; Dhama et al., 2020). People are infected with the virus but do not develop symptoms and remain asymptomatic carriers, increasing the risk of infecting the elderly and patients with underlying health conditions, leading to serious injury and death (Gasmi et al., 2021; Ma et al., 2023). Vaccines are starting to protect widespread, but the appropriate methods to prevent infection itself are basic hygiene measures such as wearing masks and hand disinfection, and there are infection methods that can be widely and easily provided to healthy people, and can be applied cheaply and prevention quickly. Furthermore, for patients with mild to moderate symptoms, it is important to suppress the spread of virus from infected people to non-infected people in order to prevent the disease from becoming more severe (Paolini et al., 2021; Abdulla et al., 2023). Furthermore, countermeasures for symptoms thought to be caused by viral infection, such as taste disturbance, fatigue, brain fog, and hair loss, have been delayed, and methods to prevent these are needed, and the world is struggling with economic leeway (Takakura et al., 2022; Tandon et al., 2024). Providing inexpensive preventive measures is also urgently needed in areas where the disease is rare. To protect widespread of virus, effective convenient materials are useful for daily lives.

Inspired by the idea that fragrances could act in the oral cavity, on the respiratory tract, or in other tissues to inhibit SARS-CoV-2 infection, the receptor-binding domain (RBD) of the SARS-CoV-2 spike protein and host ACE2. Using an evaluation system (modified ELISA method) that inhibits the binding of converting enzyme 2 (ACE2), researchers evaluated several hundred known fragrance compounds and found a candidate compound with RBD-ACE2 binding inhibitory activity (Nishimura et al., 2022). To clarify weather these flavor/fragrances

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compositions actually inhibit the infection of SARS-CoV-2, the inhibition assay using SARS-CoV-2 and VeroE6/TMPRSS2 cells were performed by the treatment by these compositions. The purpose of this commissioned research was to find new inhibitor at the cellular level of these candidate compounds, fragrances containing the candidate compounds on the infection of SARS-CoV-2 in cells.

2. Materials and methods

2.1. Fragrance compounds and mixed fragrances

The compound fragrances (Table 1) were used for the infection assay using SARS-CoV-2.

The fragrance compound and the prepared fragrance were mixed with an equal amount (volume ratio) of dimethyl sulfoxide, then appropriately diluted with phosphate buffered saline (PBS) (pH 7.4) containing 1.5 % Tween 20 and 0.5 % Bovine serum albumin (BSA), and used as a sample solution in the infection test.

2.2. Infection test

VeroE6/TMPRSS2 cells and SARS-CoV-2 mutant strains (TK8–609, E484K) were used for the test. VeroE6/TMPRSS2 cells were cultured using DMEM (low glucose) containing 2 % Fetal bovine serum (FBS), penicillin (100 units/ml), and streptomycin (100 μg/ml) as a maintenance medium at 5 % CO_2 and 37 °C. The infection test procedure is shown below. VeroE6/TMPRSS2 cells were prepared in a 48-well microplate. The number of cells were adjusted to approximately 10^4 cells/well. 1×10^7 $TCID_{50}$/2 μl of virus solution and 198 μl of sample solution were mixed and reacted at 37 °C for 20 min. A 10-fold dilution series of the reaction solution were performed with distilled water (DW), add 10 μl of the diluted solution to VeroE6/TMPRSS2 cells supplemented with 390 μl of maintenance medium, and culture them overnight. The medium was replaced with 500 μl of fresh maintenance medium and cultured for an additional 2 days. The presence or absence of cytopathic effect (CPE) in each well was observed using an inverted microscope. After fixation with formalin (for at least 30 min at room temperature), the plate was washed with DW, stained with crystal violet solution (for at least 10 min), and final judgment was made after washing with water. If CPE is suppressed by mixing the virus solution with the test substance, it will be judged as positive. $TCID_{50}$ from the number of dilutions was calculated. A strong inhibitory effect was evaluated when the minimum dilution ratio at which no CPE was observed was 100 times or more greater in the sample-added group than in the control.

2.3. Measurement of cell viability by MTT

VeroE6/TMPRSS2 cells were prepared in a 48-well microplate and cultured using DMEM (low glucose) containing 2 % FBS, penicillin (100 units/ml), and streptomycin (100 mg/ ml) at 5 % CO_2 and 37 °C. A 10-fold dilution series of 0.25 % flavor compounds, which identified as inhibiting concentration to SARS-CoV-2, were performed with DW, add 10 μl of the diluted solution to VeroE6/TMPRSS2 cells supplemented with 390 μl of maintenance medium, and culture them overnight. After removing culture supernatant, 40 μl of MTT (SIGMA CHEMICAL CO., Dorset, UK), 500 μg/ml PBS, were added on VeroE6/TMPRSS2 cells in each well of 48-well microplate and incubated at 5 %CO_2 and 37 °C. After 3–4 h, these cells were a purple color from the formal form of the cells. MTT was discarded, 100 μl dimethyl sulfoxide (Wako Pure Chemical Industries Ltd., Osaka, Japan) was added in each well. The plate is wrapped using aluminium foil, leave it for 24 h at room temperature and then read absorption of 492 nm used microplate absorbance meter (CORONA ELECTRONIC, Ibaraki, Japan).

Table 1
Flavor compounds and mixed fragrances.

Number	Compound name/sample name
A-083	Methyl anthranilate
A-086	Cinnamaldehyde
A-105	Helional
A-119	Aldehyde. C18 (γ-Nonalactone)
A-135	Cinnamyl alcohol
A-242	Dihydroactinidiolide
A-324	2-Hydroxybenzaldehyde
B-001	Cinnamon flavor, FU-8953
B-002	Cinnamon mint, FU-8954

3. Results

A total of 8 flavor/fragrances composition were conducted for the infection assay using SARS-CoV-2. Fragrance compounds excepting for cinnamyl alcohol, 0.25 % compounds, strongly inhibited the infection of SARS-CoV-2 to into VeroE6/TMPRSS2 cells (Table 2) because cinnamyl alcohol could be intermediately melted by PBS (pH 7.4) containing 1.5 % Tween 20 and 0.5 % BSA. Moreover, Dihydroactinidiolide were not completely melted and, therefore, could not be applied for the infection assay. Volume of cinnamyl alcohol is not enough to inhibit the infection of SARS-CoV-2 as compared with other fragrance compounds. Table 2 shows the results of determining the infection-suppressing effect at each concentration of the fragrance compound. The inhibition effects were lost at 0.03125 % concentration of compounds excepting for cinnamon flavor and cinnamon mint. The inhibition effects of cinnamon flavor and cinnamon mint were observed at 0.03125 % and lost at >0.03125 % concentration of compounds. To confirm that the infection-suppressing effect of the fragrances was not due to cell damage, cell viability was examined by MTT assay in a 10-fold dilution series (1/10~1/100,000) of 0.25 % flavor compounds, which identified as inhibiting concentration to SARS-CoV-2. Cultures with fragrances had absorbance values at 492 nm similar to those of the PBS-treated controls (Fig. 1). Therefore, cell viabilities were maintained in all cultures with fragrances. It was confirmed that the effect of the fragrance was to specifically suppress infection by SARS-CoV-2.

4. Discussion

Four years passed from new start of SARS-CoV-2 infection in the world. New variants are increasing for 4 years and could be not protected in the infection of virus. Over the past four years, mutant viruses of the new coronavirus have appeared one after another, and the virus that is the dominant virus has been replaced. After that, the virus continued to mutate, and in 2023, a mutant virus called "XBB", a type of Omicron strain, became the mainstream virus. Currently, a mutant virus called ``JN.1'', one of the Omicron strains, is increasing worldwide, and the World Health Organization (WHO) is monitoring it. This "JN.1″ appears to have a stronger ability to evade immunity, and as the number of infected people is increasing in some countries, there are concerns that the infection will spread again in the world.

The flavor/fragrances composition were screened from hundreds of known flavor compounds as a convenient material with RBD-ACE2 binding inhibitory activity in a previous study (Nishimura et al., 2022). They found 14 kinds of flavor/fragrance compositions that potentially inhibited vRBD-hACE2 binding (Nishimura et al., 2022). Among these flavor/fragrance compositions, Helional that had the inhibitory activities on vRBD-ACE2 binding in protein-protein and protein-cell interaction, showed strong inhibition effects on the real infection of variant, E484K of SARS-CoV-2 to VeroE6/TMPRSS2 cells in this study. This indicated that the inhibition effects in protein-protein and protein-cell interaction were reflected by the inhibition effects of real infection of virus to the cells. The infection-inhibiting effect of fragrances may be derived from the physical interactions commonly required for SARS-CoV-2 infection. These blended fragrances were also

Table 2
Infection-inhibiting effects of fragrance compounds and blended fragrances.

Number	Compound name/sample name	Inhibition effects of compounds on the infection at treatment concentrations[1)]					
		1.0 %	0.50 %	0.25 %	0.125 %	0.0625 %	0.03125 %
A-083	Methyl anthranilate	++	++	++	++	±	NT
A-086	Cinnamaldehyde	++	++	++	++	+	NT
A-105	Helional	++	++	++	++	+	NT
A-119	Aldehyde. C18 (γ-Nonalactone)	++	++	++	++	+	NT
A-135	Cinnamyl alcohol	++	++	+	NT	NT	NT
A-324	2-Hydroxybenzaldehyde	++	++	++	++	+	NT
B-001	Cinnamon flavor, FU-8953	++	++	++	++	+	+
B-002	Cinnamon mint, FU-8954	++	++	++	++	+	+

++:strongly, +:intermediately, ±:weakly, -:nothing, NT:not tested.

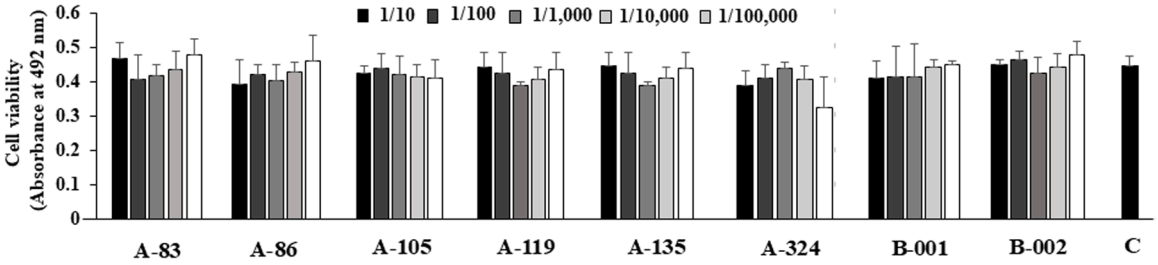

Fig. 1. Observation of cell viability in the VeroE6/TMPRSS2 cells. Cell viabilities were observed by MTT methods. A 10-fold dilution series (1/10~1/100,000) of 0.25 % flavor compounds, which identified as inhibiting concentration to SARS-CoV-2, were performed with DW, add 10 μl of the diluted solution to VeroE6/TMPRSS2 cells supplemented with 390 μl of maintenance medium, and culture them overnight. After culture, cells were treated by MTT and cell viability was measured by absorbance at 492 nm. The data indicated the mean ± SD of three independent experiments.

tested for the inhibiting activities to SARS-CoV-2 infection in this study and some samples were found to have a strong effect on inhibiting the infection of SARS-CoV-2 to VeroE6/TMPRSS2. Therefore, it is considered that foods containing these flavorings have the potential to become innovative foods that can be expected to have an infection-inhibiting effect while being eaten. It is clear that infection spreads through the oral cavity. In particular, ACE-2 is expressed in the salivary glands, which is clearly a stage for infection (Xu et al., 2020). ACE-2 is also expressed in the oral mucosa (Matuck et al., 2021; Tanaka et al., 2023), and cinnamon mint and cinnamon flavor, which are convenient food materials as mix flavor/fragrances that may inhibit infection of SRAS-CoV-2 in the oral cavity, are extremely useful for preventing spread of the infection. On the other hand, for gums containing inhibitory flavoring compounds, it is necessary to increase the amount of flavoring compounds added to the chewing gum so that a sufficient amount of the flavoring compound is eluted to suppress infection, to change the amount of the flavoring compound to be easily eluted from the chewing gum, and to develop some kind of technology. Therefore, it is necessary to improve the dissolution properties from chewing gum. It was also considered necessary to consider ingredients other than gum, such as candy and paste (jam, etc.).

In addition to the increasing number of new mutated viruses worldwide, we are also receiving a lot of consultations about the after-effects of infection, and measures to deal with the after-effects will continue to be an issue. During symptoms, aftereffects are rising as a problem to be solved after the infection. The problem with infectious diseases in daily life is that people can spread the infection without noticing it. In this study, these candidates of flavor/ fragrances in food may be helpful for development of new habits to prevent SARS-CoV-2 infection together with the after-effects in their daily lives.

5. Conclusion

The strategy of using flavor/fragrances compositions such as cinnamon flavor and cinnamon mint may be useful to protect widespread of SARS-CoV-2 and to create development of new habits in their daily lives.

Declaration of competing interest

The author declare that I have no known competing financial interests or personal relationships that could have appeared influence the work reported in this paper.

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Data availability

No data was used for the research described in the article.

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