Using SPME-GC/REMPI-TOFMS to Measure the Volatile Odor-Active Compounds in Freshly Cooked Rice

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ABSTRACT: The volatile odor-active compounds of cooked rice were evaluated using a method that combined solid-phase microextraction (SPME) with gas chromatography–resonance-enhanced multiphoton ionization time-of-flight mass spectrometry (GC/REMPI-TOFMS). An SPME fiber was held at the upper levels of the cooked rice and given an extraction time of 5 min. By using a nanosecond ultraviolet (266 nm) pulsed laser for ionization, two compounds, 4-vinylphenol and indole, which are considered to be important for the characteristic flavor of cooked rice, could be detected from all types of cultivars measured in the present study—nonglutinous rice, glutinous rice, and aromatic rice. In the case of fresh nonglutinous rice, the amounts of introduction for 4-vinylphenol and indole to GC were ca. 70 and 20 pg, respectively. While both peak areas decreased with increases in the time needed to maintain warmth, the decreasing behaviors differed slightly with a noteworthy rapid decrease for indole. For nonglutinous rice, the peak areas for 4-vinylphenol were almost the same, whether it was fresh (measured within 1 month from harvest) or aged (measured 6–12 months after harvest), but those of indole significantly decreased following storage. We also found differences among cultivars: the peak area for 4-vinylphenol in nonglutinous rice was somewhat strong; the peak area for indole was intensely strong in glutinous rice; however, the peak areas for both 4-vinylphenol and indole were weak in aromatic rice. Volatile odor-active compounds were detected in a sensitive and time-resolved manner; therefore, the proposed method could be useful for differentiating varieties of cooked rice from the viewpoints of cooking conditions, freshness, and cultivar types.

INTRODUCTION

Rice is a staple food in many countries. In Japan, novel rice cultivars are actively being developed with qualities such as taste, flavor, and texture that match the preferences of consumers. Sensory analysis of rice is subjective and is accomplished by using the human senses to evaluate the taste and flavor. To aid selection by consumers, the characteristics of rice cultivars should be objectively, for example, quantitatively, explained. For the analysis of volatile compounds from cooked rice, gas chromatography–mass spectrometry (GC/MS) is normally employed.1–3 Yang et al. identified 35 volatile compounds via GC/MS using a dynamic headspace system with Tenax trapping4 and also evaluated volatile compounds emanating from six different types of rice flavors.5 Solid-phase microextraction (SPME) can extract and concentrate trace compounds from a sample without the use of a solvent for extraction.6,7 SPME has simplicity and repeatability, which allows its use for pretreatment and simple extraction methods for GC/MS.8–10 A combination of SPME and GC/MS has been used to analyze the flavor of rice. For example, Zhen et al. extracted the volatile compounds during cooking from three nonglutinous cooked forms of rice via SPME and compared the differences of the obtained signal intensities.11 Bryant and McClung examined the volatile profiles before and after the storage of several cultivars.12 However, a quantitative evaluation of the odor of cooked rice has remained a difficult undertaking. The odor can be changed by factors such as the type of rice cultivar, the storage time, the cooking conditions, and the time following cooking. Therefore, analysis of the flavor of cooked rice, particularly at mealtime, is quite important in order to establish a subjective opinion that the cooked rice is delicious, which is the primary objective in the development of favorable rice cultivars.

Resonance-enhanced multiphoton ionization time-of-flight mass spectrometry (REMPI-TOFMS) using ultraviolet (UV) pulses for ionization is a selective analytical method.13–17

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Namely, compounds with absorption against the laser wavelength that is applied, mainly aromatic compounds, can be sensitively and selectively detected. Until now, Zimmermann et al. have reported applications in evaluating volatile flavor compounds during coffee roasting.\(^{18,19}\) Moreover, the combination of gas chromatography and REMPI-TOFMS (GC/REMPI-TOFMS) is quite useful for the selective analysis of complex real samples.\(^{20−23}\) For example, we applied this method to the measurement of the polycyclic aromatic hydrocarbons (PAHs) in aerosol particulate matters, and more than 100 peaks were confirmed.\(^{24}\)

In the present study, volatile compounds from cooked rice were extracted by SPME and were measured by GC/REMPI-TOFMS. In order to evaluate compounds at a given mealtime, volatile compounds were extracted from the upper levels of the rice via SPME with an extraction time of 5 min. The behavior of the peak areas of extracted odor-active compounds was discussed in terms of the time for maintaining warmth after cooking, the storage time, and the particular rice cultivar.

### EXPERIMENTAL SECTION

#### Rice Samples and Authentic Reagents. Three kinds of Japanese rice cultivars, non-glutinous rice (Ichihomare),\(^{25}\) glutinous rice (Tancho-mochi), and aromatic rice (Towanishi-ki) were used, all of which were harvested either in 2018 or 2019. The non-glutinous rice was cultivated at the Fukui Agricultural Experiment Station (Japan). Among the non-glutinous samples, one was milled as soon as possible (within one month after being harvested) and was cooked just after milling (called fresh rice). The other was milled ca. 5 months after being harvested and was cooked ca. 7 months after milling (called aged rice). Milled glutinous rice (cultivated in the Fukui prefecture) and milled aromatic rice (cultivated in the Kochi prefecture) all were purchased from different rice stores and were cooked ca. 4−12 months after being harvested. All rice was stored in a refrigerator until measurement. The compounds 4-vinylphenol (Alfa Aesar, CAS no. 2628-17-3, ca. 10% solution in propylene glycol) and indole (FUJIFILM Wako Pure Chemical Corporation, CAS no. 120-72-9, 98.0+%) were used to identify the peaks obtained from the measurement of volatile compounds arising from cooked rice. A standard solution of PAHs (Supelco) was routinely used for GC/REMPI-TOFMS.

#### Cooking Procedure. The washing procedure and cooking conditions of the rice were as follows. First, a certain amount of water was added to 450 g of milled rice, which was stirred lightly and then drained. Next, the rice was stirred 20 times by hand, and a certain amount of water was added and then drained. This procedure was repeated three times. The final amount of water added was 621 g (1.38 times the volume of the milled rice used) in the case of nonglutinous and aromatic rice. Cooked glutinous rice becomes pasty when the same amount of water as that listed above is used for cooking. Therefore, the final amount of water added to glutinous rice was set to 497 g, which was 80% of the before-mentioned amount of water and is standard for cooking glutinous rice boiled with red beans (sekihan in Japanese).

Rice was cooked using a household induction heating rice cooker (SR-HD103, Panasonic, Japan). The time for cooking was ca. 26 min, which is a quick-cooking mode. After cooking, the rice was allowed to stand for 5 min with the lid closed except to confirm the relationship between the obtained peak areas and the time for maintaining warmth, which was checked at 5 min, 2, 6, and 24 h. The temperature for maintaining warmth was set as 60 °C.

#### Conditioning of SPME and Extraction Conditions. A 1 cm 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane StableFlex fiber (57328-U, Sigma-Aldrich), which was equipped with an SPME holder (57330-U, Sigma-Aldrich), was used for SPME. Volatile compounds from cooked rice were adsorbed onto the fiber. Before its use for extraction, the fiber was inserted into a piped heater (TMF-300N, AS ONE) with a temperature of 270 °C for more than 1 h for preconditioning.

Figure 1 shows a schematic diagram of the experimental apparatus used in the present study. As previously mentioned, cooked rice was kept for 5 min or more before the extraction. An SPME fiber was then held at the upper levels of the cooked rice by hand for 5 min. At that point, the cooked rice was mixed twice, at 0 min (i.e., just after opening the lid of the rice cooker) and at 2.5 min, after beginning the extraction.

![Figure 1. Schematic diagram of the experimental setup.](https://dx.doi.org/10.1021/acsomega.0c03037)
where many constituents are detected. In the cases of maintaining warmth for 5 min and 1 h, fresh nonglutinous rice cooked within 1 month of harvest, smelled sweet, which is a general quality of Japanese nonglutinous rice. On the other hand, the sweet odor was reduced and an unpleasant odor was increased when the time for maintaining warmth was more than 2 h.

When maintaining warmth for 5 min, aged nonglutinous rice had a slight odor that was characteristic of rice bran. Moreover, as a reference, further aged nonglutinous rice, which was stored for 2–3 years after milling, had an unpleasant odor that was unappetizing.

Cooked glutinous rice had the odor of rice cakes (mochi in Japanese). Cooked aromatic rice had an odor that resembled popcorn, which was particularly intense during cooking. As will be developed later, 4-vinylphenol and indole were detected from the extracts of the vapor in every form of cooked rice used in the present study. We directly sampled the aroma of each authentic reagent: 4-vinylphenol had the sweet odor of caramel (like cough syrup for children); indole had the odor of an insecticide for household use, though it is said that low concentrations of indole have the odor of jasmine.

Two-Dimensional Display of Volatile Compounds in Cooked Rice. In the present study, volatile compounds in cooked rice were extracted from the upper levels of the rice via SPME with an extraction time of 5 min. The volatile compounds were then measured by GC/REMPI-TOFMS. As an example, a two-dimensional display obtained from fresh nonglutinous rice is shown in Figure 2. A few peaks appeared from the vapor of all forms of cooked rice in the present study. When a nanosecond UV laser was applied, REMPI showed superior selectivity, and constituents with a resonance wavelength for absorption could be selectively ionized. Therefore, few peaks were detected compared with that obtained using conventional GC/MS with electron ionization where many constituents are detected.

Among the peaks appearing in Figure 2, two peaks with m/z values of 120 and 117 in the order of elution were detected in every measurement in the present study. These were identified as 4-vinylphenol and indole by comparing the results of each authentic sample. The behavior of these peak areas obtained from cooked rice will be discussed in the next section. Other peaks appearing in Figure 2 were not always detected because of the low sensitivity; the use of a high repetition-rate UV laser would be effective for improving sensitivity.

Signal Behavior Against the Time Used to Maintain Warmth, the Storage Time, and the Type of Cultivar. Figure 3 shows the relationship between the time for maintaining warmth and the peak area for 4-vinylphenol and indole obtained from cooked fresh nonglutinous rice. The extraction time was 5 min, which means that highly time-resolved signal behavior could be obtained. Both peak areas were the largest when the time used to maintain warmth was 5 min, that is, the amount of both compounds in the vapor was the largest when the rice was freshly cooked. One of the tangible reasons is that the temperature was rather high just after cooking, and the amount of the released vapor was relatively large.

Zeng et al. measured the volatiles in three Japanese rice cultivars during cooking and characterized Nipponb resurrection as having a higher amount of indole, while Koshikihari and Akitakomachi were described as having higher amounts of 4-vinylphenol. In this manner, these were considered key compounds that could be used to characterize rice cultivars according to aroma. As shown in Figure 3, it was interesting that the decrease in each peak area was somewhat different. The peak area for indole rapidly decreased when the time used to maintain warmth was 2 h and then remained roughly unchanged with time; it was decreased ca. 60% at 24 h after being cooked compared with a warming time of 5 min. On the other hand, the peak area for 4-vinylphenol seemed to gently decrease with time, with a decrease ratio of ca. 40%. In our odor test (sensory analysis by nose), cooked nonglutinous rice smelled sweet for almost 2 h, after which the sweet odor was decreased and an unpleasant smell increased with time. Therefore, 4-vinylphenol and indole were not the sole
influences on the change in odor. These, and other odor-active compounds, however, exert complex influences on the change in odor.

It is worth noting that a decrease in the peak area of volatile odor-active compounds was confirmed in the present method, that is, by using a combination of SPME with an extraction time of only 5 min and GC/REMPI-TOFMS. We roughly checked the amount of each compound introduced to GC by comparing the peak areas obtained from each authentic sample. As a result, in the case of the experimental conditions shown in Figure 2, the amounts of introduction were ca. 70 and 20 pg for 4-vinylphenol and indole, respectively. In the present study, we decided on such a short extraction time because we aimed to analyze the volatile compounds in freshly cooked rice. Consequently, such a small amount of compounds and their change with time could be measured.

Figure 4 shows the peak areas of 4-vinylphenol and indole obtained from the vapor of various types of cooked rice used in the present study. From the viewpoint of the freshness of nonglutinous rice, the peak area of 4-vinylphenol was almost the same regardless of whether it was fresh or aged. Yajima et al. reported that 4-vinylphenol and 4-vinylguaiacol are formed by the thermal decomposition of the p-coumaric and ferulic acid that exists in rice during cooking and/or steam distillation, which was applied by the authors.27 Therefore, there is a possibility that the amounts of these source compounds were not changed significantly during storage. By contrast, the peak area of indole obtained from aged nonglutinous rice was much smaller than that obtained from the fresh source. As mentioned previously, aged cooked rice has an unpleasant odor. The smell of cooked rice consists of a mixture of odor-active compounds that include 4-vinylphenol and indole. Changes in the amounts of the odor-active compounds can be analyzed using the present method, which will be useful for characterizing the odor of cooked rice according to the storage time.

Figure 4. Comparison of the peak areas of volatile compounds in cooked rice (n = 3–4): (a) 4-vinylphenol; (b) indole.

Differences in the peak areas were obvious among the different types of rice cultivars. The peak area of 4-vinylphenol from nonglutinous cooked rice was higher than that from either glutinous rice or aromatic rice. Indole was strongly detected from cooked glutinous rice. Incidentally, cooked glutinous rice had the characteristic odor of rice cakes, but the smell was completely different from that of concentrated authentic indole.

In the case of aromatic rice, the peaks of 4-vinylphenol and indole were both small. Aromatic rice had the characteristic odor of popcorn, which is the corresponding odor of 2-acetyl-1-pyrroline.5 The odor threshold of 2-acetyl-1-pyrroline is quite low with respect to detection by the human nose.2 In the present study, this compound could not be detected, probably because of the optical selectivity of REMPI and/or the small quantity available for recognition by the human nose.

■ CONCLUSIONS

In the present study, SPME-GC/REMPI-TOFMS was applied to the measurement of the volatile compounds in cooked rice. In order to evaluate the odor under real conditions of cooking and eating, the extraction time was set at 5 min. As a result, even with such a short extraction time, 4-vinylphenol and indole could be selectively and time-resolvedly detected. These compounds were found to be characteristics of the differences in the time used to maintain warmth after being cooked, the storage time, and the type of cultivar. Therefore, the present method is surely useful for characterizing the odors of cooked rice, which imparts information concerning the cooking and storage conditions of rice in order to maintain a delicious image for consumers.

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Notes

The authors declare no competing financial interest.

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