Discrimination of the Cultivar, Growing Region, and Geographical Origin of Rice (*Oryza sativa*) Using a Mass Spectrometer-Based Electronic Nose

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Abstract The objective of this study was to discriminate the cultivar, growing region, and geographical origin of rice (*Oryza sativa*) using a mass spectrometry-based electronic nose (MSE-nose). The inside-needle dynamic extraction (INDEX) system was used to concentrate the samples for MSE-nose, following which the ion fragment data obtained were used to perform discriminant function analysis. Discriminant functions 1 and 2 readily separated all 16 cultivars of rice sampled. It was also confirmed that MSE-nose could distinguish the region in which rice cv. *Chucheong* and *Koshihikari* were grown, likely due to variation in environmental factors, such as soil and climate. Finally, it was confirmed that MSE-nose could be used to detect the geographical origin of rice, discrimination Korea rice from Japanese rice. Therefore, this simple and rapid technique is of value for discriminating the cultivar, growing region, and geographical origin of rice.

Keywords: rice, cultivar, growing region, geographical origin, electronic nose

Introduction

The recent liberalization of the international agricultural market has improved income levels and led to an increased interest in health and functionality. Consequently, at present, the focus is largely on the production of new rice (*Oryza sativa*) cultivars to better suit consumer tastes and enhance international competitiveness (1). There has been a steady increase in the number of breeding studies investigating the diversification of rice cultivars, and these have led to the development of glutinous rice, pigmented rice, and flavored rice cultivars, in addition to common rice cultivars, each of which contains different specific components, albeit in very small quantities (2).

Different cultivars of rice can be distinguished based on their form and components. However, external environmental conditions, such as temperature and water availability, have little effect on the composition of rice during the ripening period, making it very difficult to discriminate the geographical origin of rice (3).

A number of technologies are available for differentiating the cultivar and geographical origin of rice, the majority of which include high-performance liquid chromatography (HPLC) (4), gel electrophoresis (5), gas chromatography-mass spectrometry (GC-MS) (6), and inductively coupled plasma mass spectrometry (ICP-MS) (7) analyses. However, HPLC is a time-consuming technique and thus cannot be used to

quickly determine the differences between cultivars; moreover, gel electrophoresis is not only time consuming but also presents challenges in the quantification and interpretation of the bands produced (8), and GC is both time-consuming and expensive, with the added disadvantage of testing errors.

Electronic nose (e-nose) analysis is a quick and easy method that does not require pretreatment of the samples. Furthermore, this technique can detect even small changes in sample components and can rapidly predict the degree of difference between the samples. Inside-needle dynamic extraction (INDEX) can be used to concentrate the volatile components in the purified sample sufficiently for MSE-nose analysis, allowing small differences to be detected. It has the added advantage of high analysis speed and reproducibility. This system involves two steps: first, the adsorbent material is loaded onto the needle by suction, following which the sample is stored in the needle until analysis, and second, the volatile component is desorbed by heating it with a gas or air injection.

Lee and Kim (9) previously compared the volatile components contained in rice cv. Dongjinbyeo and Hyangnambyeo with brown rice and found that these cultivars contained more aldehydes and alcohols with low numbers of carbon atoms than other rice. Song *et al.* (10) also analyzed the headspace volatile patterns of 44 japonica rice cultivars using a metal oxide sensor (MOS) e-nose system and

found that it was able to distinguish 39 cultivars based on their aromatic components. Therefore, the domestic cultivars of rice can be distinguished using this technology. However, no research has been conducted to determine whether the region in which rice is grown can be determined based on the analysis of headspace volatile components. Furthermore, the first-generation MOS sensor used in e-nose has some limitation in terms of reproducibility and accuracy (11).

The aim of this study was to use an MSE-nose to identify cultivar and regional differences in Korean rice cultivars based on their volatile components. Rice cultivars originating from Korea and Japan were also analyzed; however, the initial analyses were unable to clearly distinguish the country of origin due to low concentrations of volatile compounds. Therefore, the INDEX system was implemented, which allows the trace amounts of the components to be concentrated by 10–15 times, allowing the origin of the rice samples to be successfully distinguished.

Materials and Methods

Samples Sixteen domestic cultivars of rice that had been harvested in 2014 were used in this study (Table 1). These were collected from the National Agricultural Products Quality Management Service, Gimcheon, Korea, and from commercial mart, Seoul, Korea.

To compare the volatile components of cultivars grown in different regions, rice cv. *Chucheong* grown in eight different regions and rice cv. *Koshihikari* grown in six different regions were used (Table 1). To compare the volatile components of cultivars originating from different countries, six samples of cv. *Koshihikari* grown in Korea were compared with six samples grown in Japan; and nine Korean cultivars were compared with nine Japanese cultivars (Table 1).

INDEX system Headspace volatile was concentrated by inside needle dynamic extraction (INDEX). A 1.2 g sample was placed in a 10 mL vial (La-Pha-Pack® GmbH, Langerwehe, Germany), which was then sealed using a polytetrafluoroethylene (PTFE)/silicone cap (Pharma-Fix, Chemmea, Slovakia). This was then agitated at 350 rpm for 10 min at 90°C prior to injection into the needle at an injection opening temperature of 200°C. The volatile compounds were forced through the needle by repeated aspiration/ejection motions of the syringe plunger. The sample was concentrated by 10 times of pumping syringe inside the needle, which contained silox (Hamilton, Bonaduz, Switzerland). The concentrated sample was injected to MSE-nose and air was used for a control. Measurement was repeated three times. And static headspace method principle, performance and operating procedure of the apparatus are the same as our previous works (12).

Electronic nose analysis For the headspace analysis, 2.5 mL of volatile component was collected after a 300-ms syringe purge and

Table 1. Cultivars and growing regions of the rice samples used in this study

	Cultivar	Cultivated sites
A	<i>Odae</i>	Cheorwon, Gangwon
B	<i>Samkwang</i>	Dangjin, Chungnam
C	<i>Haiami</i>	Hwaseong, Gyeonggi
D	<i>Koshihikari</i>	Jeongeup, Jeonbuk
E	<i>Hitomebore</i>	Haenam, Jeonnam
F	<i>Milky queen</i>	Goheung, Jeonnam
G	<i>Unkwang</i>	Cheorwon, Gangwon
H	<i>Sindongjin</i>	Buan, Jeonbuk
I	<i>Chucheong</i>	Sancheong, Gyeongnam
J	<i>Samdeog</i>	Kyeongju, Gyeongbu
K	<i>Jinsang</i>	Hwaseong, Gyeonggi
L	<i>Younghojinmi</i>	Andong, Gyeongbuk
M	<i>Ilmi</i>	Hampyeong, Jeonnam
N	<i>Daeon</i>	Yangju, Gyeonggi
O	<i>Hopyung</i>	Hampyeong, Jeonnam
P	<i>Saenuri</i>	Anseong, Gyeonggi
I2	<i>Chucheong</i>	Kyeongju, Gyeongbu
I3	<i>Chucheong</i>	Icheon, Gyeonggi
I4	<i>Chucheong</i>	Gimje, Jeonbuk
I5	<i>Chucheong</i>	Wonju, Gangwon
I6	<i>Chucheong</i>	Yeoju, Gyeonggi
I7	<i>Chucheong</i>	Ganghwa, Incheon
I8	<i>Chucheong</i>	Anseong, Gyeonggi
D2	<i>Koshihikari</i>	Pyeongtaek, Gyeonggi
D3	<i>Koshihikari</i>	Icheon, Gyeonggi
D4	<i>Koshihikari</i>	Gimcheon, Gyeongbuk
D5	<i>Koshihikari</i>	Yongin, Gyeonggi
D6	<i>Koshihikari</i>	Ganghwa, Incheon
D7	<i>Koshihikari</i>	Gimcheon, Gyeongbuk
1	<i>Koshihikari</i>	Shimanenita
2	<i>Koshihikari</i>	Tottorikofu
3	<i>Koshihikari</i>	Nagano
4	<i>Koshihikari</i>	Hyogotajima
5	<i>Koshihikari</i>	Niigatauonuma
6	<i>Koshihikari</i>	Niigata
7	<i>Koshihikari</i>	Niigatauonuma
8	<i>Akitakomachi</i>	Akita
9	<i>Koganemochi</i>	Niigataminamiuonuma

A-P: Different cultivar of rice in Korea

I2-I8: *Chucheong* cultivar with different cultivated sites in Korea

D2-D7: *Koshihikari* cultivar with different cultivated sites in Korea

1-9: Japanese rice with different cultivated sites in Japan

was placed on a thermostat-controlled tray holder (CombiPAL; CTC analytics, Zwingen, Switzerland). Then, the headspace system was used to analyze the gas components of the sample using an e-nose system (SMart Nose300; SMart Nose Inc., Marin-Epagnier, Switzerland) with an auto-sampler (CombiPAL; CTC Analytics) and a mass spectrometer (Quadrupole Mass Spectrometer; Balzers Instruments, Masin-Epagnier, Switzerland). The volatile substances were ionized at 70 eV, following which the ionized substances that formed over a period of 180 s were passed through a quadrupole mass filter. The substances that had a molecular weight of 10–200 u in integer units

were then passed through the mass spectrometer, and these were also used as the number of channels. The air sample from the early stages of the analysis was used as a control, and the test was repeated three times for each sample. The sensitivity of each channel was recorded in the form of a matrix, and 10-20 variable groups that exhibited the greatest differences in molecular weight among the ionized molecules were selected for discriminant function analysis (DFA) using the SMart Nose[®] statistical analysis software (Version 1.51; THOPAS Soft Creation, Marin-Epagnier, Switzerland).

Discriminant function analysis (DFA) DFA was used to detect the differences between the volatile fragrance components of rice samples that demonstrated a large difference (in the range of 10-200 u) in ion fragments in the e-nose analysis. The largest 30 samples (based on m/z values) were selected for analysis, using the following equation.

$$\text{DFA} = B_0 + B_1X_1 + B_2X_2 + B_3X_3 + \dots + B_nX_n$$

where B_0 is a constant value, B_{1-n} is a coefficient, and x is the sensitivity of each value in u. The discriminant function of the independent variables is plotted two-dimensionally, with DF1 (the first score from the DFA) along the x-axis and DF2 (the second score from the DFA) along the y-axis, both of which affect the dependent variable.

Results and Discussion

Sensitivity of INDEX system for headspace volatiles Several types of volatile components were generated from the rice samples, but only in very small quantities. The traditional e-nose technology is capable of analyzing concentrations as low as 10 ppb. However, this is not sufficient to determine the geographical origin of rice due to the very small concentrations present. INDEX is also a preconcentration technique (13). The INDEX needles contain an absorbing polymer phase very much like a fixed bed. The volatile compounds were concentrated by repetition aspiration and ejection motions of the syringe plunger. The potential advantage of INDEX system compared to solid-phase microextraction is its mechanical robustness and the possibility of increasing the amount of absorbing polymer as well as the surface area available for adsorbing volatile compounds.

The INDEX system was able to concentrate the volatile components in the samples, allowing for subtle differences to be detected. In addition, this system had the advantage of high analysis speed and reproducibility.

The mass spectra of rice, a static headspace system, and an INDEX system syringe analyzed under the same conditions are shown in Fig. 1. The e-nose measured components in the range of 10-200 u (m/z); however, as ion fragments <40 u contained were very similar components to air in terms of the molecular weight, the analysis of fragments in the range of 40-100 u most clearly demonstrated the

differences in the sensitivity of these techniques.

The mass spectrum of the samples using the static headspace system was not substantially different from the spectrum of air particles. By contrast, the INDEX system was highly sensitive to ion fragments within the range of 40-100 u, generating increased differentiation of the sample by the concentration effect.

Therefore, because environmental factors, metabolic components, and headspace volatile components show only marginal differences between rice cultivars and regions of origin, the INDEX system is a useful approach for discriminating between them.

Volatile component difference according to different cultivars of rice Sixteen cultivar of rice cultivated in Korea (A-P) as shown in Table 1 were analyzed using an MSE-nose, following which a discriminant function analysis was performed, the results of which are shown in Fig. 2A. Both scores of the discriminant functions were significant (DF1: $r^2=0.9997$, $F=7,986.90$; DF2: $r^2=0.9987$, $F=1,678.10$), and the values of DF1 and DF2 were differentiated at a ratio of approximately 5:1. Thus, the cultivars of rice were divided by the direction as well as the values of both DF1 and DF2.

The rice samples were positioned to the left of the air samples along DF1, indicating that rice contains more volatile compounds than air. However, the level of differentiation differed between cultivars, with cultivars L and D further to the left along DF1, indicating relatively high levels of volatile compounds, and cultivar J lying to the right of the plot fairly close to air, indicating the lowest levels of volatile compounds. Thus, different cultivars of rice could be distinguished based on their volatile compound contents, which varied due to differences in the metabolic activity of the cultivars.

Besides of the distinguished samples (B, D, H, and L) the rest of samples were performed again by DFA to find out whether samples were discriminate well or not. Twelve of the the sampled cultivars (A, C, E, F, G, I, J, K, M, N, O, and P) contained similar volatile compounds, making it difficult to discriminate between them. Therefore, a second DFA was performed to separate these cultivars (Fig. 2B). For this analysis, both of the discriminant functions were again significant (DF1: $r^2=0.9976$, $F=903.31$; DF2: $r^2=0.9958$, $F=508.14$) and were differentiated at a ratio of approximately 2:1. Thus, this second analysis allowed these cultivars to be differentiated based on their volatile components. When performing a DFA, it is how to select the two-dimensional pattern to distinguish.

Morphological analyses and physicochemical analytical methods have a limited ability to separate rice cultivars, therefore, new techniques require greater precision (14). Furthermore, similar rice cultivars do not exhibit a large difference in their chemical composition (15), making it difficult to distinguish between them. Kwon and Cho (16) previously applied an image processing technique to identify rice cultivars but found that this approach had limited applicability for cultivars that were very similar to each other. Rhyu *et al.* (17) used capillary electrophoresis analysis to distinguish between 23 cultivars of white rice and obtained a characteristic peak for some, including

Fig. 1. Mass spectra of air and the volatile components in rice using a static headspace system and an INDEX syringe

Dongjinbyeo; however, these peaks had poor resolution and were not reproducible, and it was difficult to obtain a pattern of peaks that allowed each cultivar to be distinguished.

The present study demonstrated that the MSE-nose technique can be used to clearly separate the volatile components of each rice cultivar, allowing the determination of the exact cultivar.

Volatile component difference according to different region of rice

Since climate and soil are considered to affect the volatile compounds produced by rice, e-nose technology should be able to determine the region of rice cultivation. Therefore, the volatile components of rice cv. *Chucheong* from eight different regions were compared using MSE-nose analysis, following which a DFA was performed, the results of which are shown in Fig. 3A. Both of the discriminant functions were significant (DF1: $r^2=0.9647$, $F=61.41$; DF2: $r^2=0.9247$, $F=27.64$), and the values of DF1 and DF2 were differentiated at a ratio of approximately 2:1.

Samples that were obtained from sites within the Gyeonggi region had similar volatile components in terms of DF1 due to the similar climate exposure. However, for the other growing regions, the volatile components were dispersed along both DF1 and DF2. Thus, it was confirmed that the volatile components of rice cv. *Chucheong* could be used to distinguish the growing region.

Other cultivars also showed different volatile component patterns between growing regions. Therefore, rice cv. *Koshihikari* grown in six different regions was also analyzed to determine whether it was possible to distinguish the growing region based on the volatile components (Fig. 3B). In this analysis, both the discriminant functions were significant (DF1: $r^2=0.9946$, $F=430.68$; DF2: $r^2=0.9767$, $F=97.96$), and the values of DF1 and DF2 were differentiated at a ratio of approximately 4:1. Thus, the growing region of the *Koshihikari* cultivar was more readily distinguished based on the volatile components than the region of the *Chucheong* cultivar.

It has previously been demonstrated that a particular cultivar of rice can exhibit variation in its chemical composition depending on its geographical origin; this is because of the influence of the amount of solar radiation, duration of sunshine, and temperature (18). The protein content of rice significantly differs between cultivars and production areas in Korea (19). Furthermore, in the case of japonica rice, the pulverized rice, fractured rice, and protein content not only differed greatly between regions but also between production years, which could have an impact on all rice cultivars and growing areas included in previous studies. Similarly, the present study showed that the volatile components in a particular rice cultivar can vary between production regions, allowing these to be determined using MSE-nose technology.

Fig. 2. Discriminant function analysis of the data obtained from a mass spectrometry-based electronic nose for (A) all 16 rice cultivars sampled (DF1: $r^2=0.9997$, $F=7986.90$; DF2: $r^2=0.9987$, $F=1678.10$) and (B) 12 rice cultivars that were not clearly distinguished in the first analysis (DF1: $r^2=0.9976$, $F=903.31$; DF2: $r^2=0.9958$, $F=508.14$).

Volatile component difference according to different geographical origin of rice

As demonstrated above, the volatile components of rice differ between cultivars. However, if the same cultivar is grown in different countries, the metabolites and headspace volatile ingredients would also be expected to differ due to variation in local environmental factors, such as soil and climate. Therefore, to determine whether the volatile components can also be used to determine the country of origin, the difference in the volatile components in six samples of rice cv. *Koshihikari* grown in Korea were compared with six samples grown in Japan using MSE-nose analysis followed by DFA (Fig. 4). Both of the discriminant functions were significant (DF1: $r^2=0.9717$, $F=617.71$; DF2: $r^2=0.8936$, $F=151.21$), and the values of DF1 and DF2 were differentiated at a ratio of approximately 4:1. Thus, domestic and Japanese *Koshihikari* rice were clearly separated along DF1. Furthermore, the volatile components of domestic rice were similar to air, while Japanese *Koshihikari* rice was a long distance to the left of air along DF1, indicating that the *Koshihikari* rice grown in Japan contains more volatile compounds than that grown in Korea.

To determine whether the geographical origin of different cultivars of rice could be distinguished according to their volatile components, this analysis was repeated using nine domestic cultivars and nine Japanese cultivars (Fig. 5). This analysis showed that both discriminant functions were significant (DF1: $F=251.37$, $r^2=0.9030$; DF2: $F=$

Fig. 3. Discriminant function analysis of the data obtained from a mass spectrometry-based electronic nose for (A) rice cv. *Chucheong* cultivated in eight different growing regions (DF1: $r^2=0.9647$, $F=61.41$; DF2: $r^2=0.9247$, $F=27.64$) and (B) rice cv. *Koshihikari* cultivated in six different growing regions (DF1: $r^2=0.9946$, $F=430.68$; DF2: $r^2=0.9767$, $F=97.96$).

176.70, $r^2=0.8675$) and that the values of DF1 and DF2 were differentiated at a ratio of approximately 1.4:1. The DFA plot revealed that all cultivars were affected by both DF1 and DF2 (Fig. 5). As was seen for rice cv. *Koshihikari*, the same cultivars of rice that originated from different geographical regions could be clearly distinguished according to their DF1 values (Fig. 5). However, several different cultivars that originated from the same region showed relatively small differences in DF1 value.

Cheajesadagul *et al.* (20) previously used a high-resolution inductively coupled plasma mass spectrometry (HR-ICP-MS) to discriminate the origin of 36 types of rice, based on a principal component analysis or discriminant analysis of the concentration of B, Co, Sr, Mo, and 21 other elements. Using this approach, they were able to determine the country of origin but found it more difficult to distinguish between rice that was grown in the northern and northeastern regions. However, the ICP method is likely to deteriorate or destroy the sample during pre-treatment, and is also time-consuming due to the analysis of relatively large elements.

In another study, Kim *et al.* (21) used Raman spectroscopy to determine the origin of rice. Rice has a lower protein content and tends to present a higher variability of chemical components. Therefore, the origin of rice could be determined using these characteristics. However, because only a very small amount of each

Fig. 4. Discriminant function analysis of the data obtained using a mass spectrometry-based electronic nose for rice cv. *Koshihikari* originating from Korea and Japan (DF1: $r^2=0.9717$, $F=617.71$; DF2: $r^2=0.8936$, $F=151.21$).

Fig. 5. Discriminant function analysis of the data obtained using a mass spectrometry-based electronic nose for nine cultivars of rice originating from Korea and nine originating from Japan (DF1: $r^2=0.9030$, $F=251.37$; DF2: $r^2=0.8675$, $F=176.70$).

sample was analyzed, there was a high level of measurement error. Finally, Suzuki *et al.* (22) used image analysis to distinguish between the origin and cultivars of rice. However, this approach was unsuccessful.

Electronic nose analysis is a non-destructive method and can be used with a small sample volume. It is also a simple technique that can analyze a large number of samples quickly. INDEX system could concentrate the samples for MSE-nose, even though it contains a very small amount of specific volatile compounds. It was used for analysis of MSE-nose and ion fragment data obtained from MSE-nose were used to perform DFA. It was confirmed that MSE-nose could be used to detect the geographical origin of rice, discrimination Korea rice from Japanese rice. Therefore, this simple and rapid technique is of value for discriminating the cultivar, growing region, and geographical origin of rice cv. *Chucheong* and *Koshihikari*.

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