Title: Identification of coumarin-enriched Japanese green teas and their particular flavor using electronic nose

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Running title: Identification of coumarin-enriched Japanese green teas using E-nose

Title: Identification of coumarin-enriched Japanese green teas and their particular flavors using electronic nose

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Abstract
Conventionally, tea flavor is analyzed through the use of a combination of gas chromatography-mass spectrometry and human taste panel. These methods present time-consuming or inaccurate factor. In this work, a rapid, accurate and nondestructive approach was put forward to identify coumarin-enriched Japanese green teas and evaluate their particular flavors using electronic nose (E-nose) technique. The multivariable analyses including principal components analysis and cluster analysis were applied to distinguish the tea samples and evaluate the particular (coumarin-like) flavor of coumarin-enriched tea under

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different infusion conditions. The correct classification was achieved for the seven tea samples with different content of coumarin. The E-nose successfully characterized the drying temperature-dependently trend of coumarin content during the manufacture process of coumarin-enriched green tea. It also revealed that the comparatively low-infusion temperature and long-infusion time were favorable for the emission of coumarin-like flavor of the tea infusion. In addition, a comparatively newly developed “absolute value expression” (AVE) analysis was employed to divide the tea flavors into quality and express them numerically. Using AVE, the role of coumarin in the total flavor of coumarin-enriched green tea was elucidated. These results suggest that E-nose could be employed to identify the green teas with particular flavor and evaluate the tea flavor.

**Keywords:** Electronic nose; Green tea; Coumarin; Flavor; Tea infusion

**Introduction**

Green tea is the most popular beverage in Japan. This high acceptability is due to many factors, and one of the most important reasons is its characteristic flavor. The conventional evaluation of tea flavor is performed through the use of a combination of gas chromatography-mass spectrometry (GC-MS) and human taste panel. Although GC-MS is an efficient chromatographic technique for identification of odors in plant or food extracts, it also presents a time-consuming factor. The sensory profiling by human taste panel is affected by external factors and usually inaccurate because of a lack of either sensitivity or quantitative information (*Dutta et al., 2003a*). Aroma extract dilution analysis technique is also a useful approach to determine the potency of odors in tea (*Kumazawa and Masuda, 2002*), but it requires the combination with the GC analysis and GC-olfactometry technique. Recently,
electronic nose (E-nose) instruments, which mimic the olfactory receptor in human nose, have been developed that allow highly sensitive, increasingly fast, reliable odor analyses and hence represent promising tools for continuous real time monitoring of odors of food and beverage. E-nose devices have been successfully applied to different fields particularly in food and beverage industries, such as snake fruit (Supriyadi et al., 2004), tomato (Hernández Gómez et al., 2008), and coffee (Falasconi et al., 2005). E-noses have also been used for identification of tea grade (Yu et al., 2008), prediction of tea quality (Dutta et al., 2003b), and monitoring of black tea fermentation process (Bhattacharyya et al., 2007). However, little information is available regarding the application of E-nose in identification of teas with particular flavor and evaluation of tea flavor.

In our previous study, several Japanese green teas were found to have particular sweet-herbaceous fragrance by the sensory evaluation (Yang et al., 2008). Furthermore, application of GC-MS for volatile analysis revealed that these green teas with sweet-herbaceous odor contained comparatively high content of coumarin, a character impact compound for the sweet odor quality of Japanese green tea, so called “coumarin-enriched” Japanese green tea” (Yang et al., 2008). The objective of this study is to investigate whether it is possible to develop a rapid, accurate, and nondestructive E-nose-based approach to distinguish the Japanese green teas with different content of coumarin and evaluate the role of coumarin in the flavor of coumarin-enriched green tea.

Materials and methods

Tea sample
The seven Japanese green tea products (the first crop, 2007) including “Shizu-7132”, “Koushun”, “Kanayamidori”, “Yabukita”, “Macikuo”, “Okunosawa”, and “Okumidori” were purchased from the market in Japan. GC-MS analysis showed that the concentrations of coumarin in common green tea products were generally below 0.2 μg/g, whereas “Shizu-7132” and “Koushun” contained 0.88 and 0.67 μg/g coumarin respectively, so called coumarin-enriched green teas (Yang et al., 2008).

The previous study also indicated that coumarin content was drying temperature-dependently reduced during the manufacturing process of coumarin-enriched Japanese green tea “Shizu-7132” (Yang et al., 2008). To investigate the discriminating ability of E-nose for different manufacture processes of tea sample, sampling from different drying temperature (50 °C, 60 °C, 70 °C, and 80 °C) was carried out for E-nose analysis.

Sample preparation for E-nose analysis

Tea sample equivalent to 1 g of dry tea product was infused with 50 ml fresh boiled deionized water for 3 min and filtered using the tea filter paper (Daiso Industries, Ltd., Japan). The filtrate was immediately cooled to about 25 °C in trap water. 3 mL of the tea infusion was injected into a special gas bag (2 L, FF-1, 2KF, Shimadzu, Japan) used for odor analysis, and then filled with the nitrogen. To stabilize the concentration of odors in the headspace of the bag, the gas bag with tea infusion and nitrogen was kept for 2 h at room temperature in dark and then analyzed by the E-nose.

To select the optimum infusion conditions of the coumarin-enriched green tea (Shizu-7132) for the particular (coumarin-like) flavor, a mixed level design was conducted using a two-variable and three-level (infusion time: 40 s, 160 s and 280 s; infusing temperature: 60 °C, 75 °C and 90 °C) based on SAS software (Ver. 8.0, SAS, Inc). The flavors of nine treatments
were compared with that of the tea sample blended with coumarin (1 ppm) by E-nose analysis.

E-nose and measuring condition

The E-nose device (FF-2A Fragrance & Flavor Analyzer, Shimadzu, Japan) was equipped with an array of 10 different oxide semiconductor sensors, trap tube and CPU (Fig. 1A). The headspace tea aroma was pumped into the sensor chamber for 60 sec at a flow rate of 165 ml/min, and analyzed by the sensors within two measuring modes (Fig. 1B). Combination of the two measuring modes can reflect the more characters of the sampling odors. Using the trap tube, the sensitivity, reproducibility and distinctiveness of measurement are increased because of the concentration of sampling odor and the removal of humidity in sampling odor.

Pattern recognition of E-nose

Multivariate analyses including principal components analysis (PCA) and cluster analysis (CA) were applied to distinguish the tea samples and evaluate the particular (coumarin-like) flavor of the coumarin-enriched tea under different infusion conditions. All the calculations were performed by SPSS software (Ver. 14.0, SPSS, Japan Inc.).

A comparatively newly developed “absolute value expression (AVE)” controlled by ASmell2 software (Ver. 1.09, Shimadzu, Japan) was employed to divide the tea aroma into quality and express them numerically. As shown in Fig. 2, the concept of vector is introduced into the representation of odor. The direction of vector presents the quality of odor. The similarity indices to standard odors are put forward to express the quality of odor numerically.

Results and discussion

E-nose response to tea aroma and feature extraction for the classification analysis
As shown in Fig. 3, when estimating the sensor response to a given tea sample (Shizu-7132), the response values were used as $R = -\log (R_x / R_b)$, where $R$ was the response, $R_b$ the baseline resistance of a sensor (without sampling odor), and $R_x$ was the measured resistance of a sensor with sampling odor. Both measuring modes (Fig. 1B) showed different characteristics. The trap tube can remove the humidity in the aroma of tea fusion and concentrate the sampling aroma. Consequently, the signals of some sensors in the measuring mode with trap tube appear to be stronger than that without trap tube. This may imply that the humidity in the sampling odor can affect the measurement of the sensors. Although some applications of E-nose to the teas have been reported, none is involved in the effect of sample humidity on the sensor analysis. To characterize the sampling tea aroma, combination of the responses of sensors within both measuring modes was used in this work.

Feature extraction of sensor response is of great importance, which requires the conversion of sample features to patterns that have condense representations, ideally containing only salient information (Yu et al., 2008). In this work, the maximum values of the response of each sensor within the two measuring modes were extracted for the latter classification analysis (PCA and CA).

Identification of coumarin-enriched green teas and optimization on the tea infusion conditions for the coumarin-like flavor using PCA and CA

The number of data per sample was 20 (maximum values of the responses of 10 sensors within the two measuring modes). PCA was employed to reduce the dimensionality and visualization of datasets while retaining as much as possible of the variation present in the original dataset. In this work, the dimensionality of the datasets obtained by the E-nose was reduced to three dimensions due to the score of the first three-component (PC1, PC2, and PC3). The
three-dimension scatter plot of PCA for the seven tea samples is shown in Fig. 4A. It can be found that the cumulative reliabilities of PC1, PC2, and PC3 are 95.754%, which appear to provide the enough information for the best discrimination of the teas. Fig. 4A reveals that the seven tea samples were well-separated. Additionally, the coumarin-enriched green teas (Shizu-7132 and Koushun) were clearly distinguished from the green tea group with less coumarin. CA was also employed to examine the sensorial data (maximum values of sensor response) and to test the relationships of various tea groups. In the present study, the measurement of similarity is based on the pearson correlation and the cluster method is the between-groups linkage. The position of the line on the scale indicates the distance at which clusters are joined. The observed distances are rescaled to fall into the range of 1 to 25 by SPSS software (Fig. 4B, 5B, and 6B). The ratio of the rescaled distances within the dendrogram is the same as the ratio of the original distances. Fig. 4B shows that the cluster including coumarin-enriched green teas (Shizu-7132 and Koushun) was obtained, which is in good accordance with the PCA result. It is generally accepted that E-noses are capable of distinguishing the different kinds of tea samples because of the high sensitivity. The results presented here imply that E-nose also possessed the ability of classification of the tea flavor. Fig. 5 reveals the drying temperature-dependently trend of coumarin content in coumarin-enriched green tea (Shizu-7132), suggesting that E-nose can be employed as a tool for monitoring the characteristic aroma during the manufacture process of tea.

It is well known that the flavors of tea infusions vary with the infusing temperature and time. To evaluate the coumarin-like flavor of coumarin-enriched tea under different infusion conditions, we also compared the E-nose response signals of tea aroma under the 9 different infusion conditions with that of the tea sample blended with coumarin (1 ppm) using PCA and
Fig. 6 reveals that the response signals of E-nose for the tea infused with 60 °C water for 280 s and the treatment with coumarin are much closer. This indicates that the comparatively low-infusion temperature and long-infusion time were favorable for the emission of coumarin-like flavor of tea infusion.

Evaluation of the role of coumarin in the total flavor of coumarin-enriched green tea

Although coumarin-enriched green teas contain comparatively high content of coumarin, it remains to be determined for the role of coumain in the total tea flavor. In this study, the AVE method was employed to express the quality of tea aroma numerically (Fig. 2). The coumarin-enriched green teas (Shizu-7132 and Koushun) and green tea with less coumarin (Yabukita) were defined as the standard odors. The standard mode was accomplished by ASmell2 analysis software (Ver. 1.09, Shimadzu, Japan). As shown in Fig. 7, the similarity index of the total flavor of Yabukita to that of Shizu-7132 and Koushun increased after the treatment with coumarin equivalent to the content in Shizu-7132 and Koushun. This suggests that coumarin plays an important role in the total flavor of coumarin-enriched green teas.

Conclusion

GC-MS has been extensively applied to the studies on tea flavor. However, GC-MS requires the further extraction with organic solvents for separating the odorant from nonvolatile matrix compounds. The present study confirmed the merits of E-nose in respect to sensitivity and accuracy. Once more, the simple and fast sample preparation without the organic solvents points to the superiority of the E-nose. Using E-nose, the identification results of coumarin-enriched green teas were satisfied (Fig. 4). E-nose was also suitable for monitoring
the characteristic aroma during the tea manufacture process (Fig. 5) and evaluating the particular flavors under the different infusion conditions (Fig. 6). The numerical expression of the odors was introduced into E-nose (Fig. 7), making it more applicable for the evaluation of tea flavor. The highly sensitive, ultrafast, and specific techniques would be desirable to evaluate the flavors in food, which will effectively improve the quality control of food and beverage.

Abbreviations

AVE, absolute value expression; CA, cluster analysis; E-nose, electronic nose; GC-MS, gas chromatography-mass spectrometry; PCA, principal components analysis.

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References


Legends to figures:

Figure 1 The schematic diagram of electronic nose (FF-2A Fragrance & Flavor Analyzer, Shimadzu, Japan) (A), and its measuring conditions (B).

(A) The E-nose (FF2A) is equipped with 10 different oxide semiconductor sensors positioned in a chamber. FF2A system consists of a sampling apparatus, trap tube, a detector unit containing the array of sensors, and computer program (CPU and PC) for data recording and analysis. The headspace tea aroma was pumped into the sensor chamber and the response value of each sensor changed. The measurement procedure was controlled by the computer program.

(B) The sampling tea aroma was pumped straightforwardly to the sensor chamber not via trap tube and measured by the sensors (Measuring mode I). The sampling tea aroma was conducted to the trap tube for the removal of the humidity (Drying purge), concentrated by the trap tube, and then measured by the sensors (Measuring mode II).

Figure 2 Principle of absolute value expression (AVE) controlled by ASmell2 software (Ver. 1.09, Shimadzu, Japan).

The response signals of 10 sensors were represented by vector of 10 dimensions. The direction of the vector shows the quality of odor. The similarity index between sampling odor and standard odor is expressed by the angle between their vectors.

Figure 3 Response curves of 10 sensors (ch1, ch2, ..., ch10) within two measuring modes to
the aroma of the green tea (Shizu-7132) infusion

The odors in the headspace of gas bag was pumped into the E-nose for 60 s, and analyzed by 10 sensors within two measuring modes. Measuring time of the sensors in each mode was 120 s.

Figure 4 PCA (A) and CA (B) for E-nose response to the aroma of the Japanese green tea infusions.

Analytical results are based on the maximum values of the response of each sensor within the two measuring modes.

Figure 5 PCA (A) and CA (B) for E-nose response to the aroma of sampling from different drying temperature (50 °C, 60 °C, 70 °C, and 80 °C) during the manufacturing process of coumarin-enriched green tea (Shizu-7132).

Analytical results are based on the maximum values of the response of each sensor within the two measuring modes.

Figure 6 PCA (A) and CA (B) for E-nose response to the aroma of the coumarin-enriched green tea (Shizu-7132) under the different infusing conditions.

Analytical results are based on the maximum values of the response of each sensor within the two measuring modes.
Figure 7 Comparison of similarity indices to coumain-rich or weak green teas as standards between the tea sample (Yabukita) and its treatment with coumarin.
Fig. 1 Ziyin YANG
Ten dimensions of odors space based on the response signals from 10 sensors

Vector representation:
- Direction of vector: The quality of odor

Standard odor (known odor)

Similarity index

Sampling odor

θ = 0, similarity 100%
θ > a, similarity 0%
(a, the angle automatically controlled by the system)

Fig. 2 Ziyin YANG
Fig. 3 Ziyin YANG
Coumarin-enriched green teas

3-dimension plot of PCA

Dendrogram using average linkage (between groups)

Dendrogram of hierarchical cluster analysis

Fig. 4 Ziyin YANG
Fig. 5 Ziyin YANG

A

3-dimension plot of PCA

B

Dendrogram using average linkage (between groups)

Dendrogram of hierarchical cluster analysis
**A**

3-dimensional plot of PCA

- Rescaled distance cluster combine

**B**

Dendrogram using average linkage (between groups)

Tea+coumarin (1 ppm)

Dendrogram of hierarchical cluster analysis
Green tea with less coumarin
(0.09 μg/g)

Yabukita

Green tea with comparatively high content of coumarin
(0.88 μg/g)

Similarity index (%)

Yabukita

90
80
70
60
50
40
30
20
10
0

Yabukita + coumarin (0.8 μg/g) (n=3)

Shizue-7132

Koushun

Green tea with comparatively high content of coumarin
(0.67 μg/g)

Fig 7 Ziyin YANG