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Feasibility of using a bacteriophage-based structural color sensor for screening the geographical origins of agricultural products

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ABSTRACT

An M13 bacteriophage-based color sensor, which can change its structural color upon interaction with a gaseous molecule, was evaluated as a screening tool for the discrimination of the geographical origins of three different agricultural products (garlic, onion, and perilla). Exposure of the color sensor to sample odors induced the self-assembled M13 bacteriophage bundles to swell by the interaction of amino acid residues (repeating units of four glutamates) on the bacteriophage with the odor components, resulting in a change in the structural color of the sensor. When the sensor was exposed to the odors of garlic and onion samples, the RGB color changes were considerable because of the strong interactions of the odor components such as disulfides with the glutamate residues on the sensor. Although the patterns of the color variations were generally similar between the domestic and imported samples, some degrees of dissimilarities in their intensities were also observed. Although the magnitude of color change decreased for perilla, the color change patterns between the two groups were somewhat different. With the acquired RGB data, a support vector machine was employed to distinguish the domestic and imported samples, and the resulting accuracies in the measurements of garlic, onion, and perilla samples were 94.1, 88.7, and 91.6%, respectively. The differences in the concentrations of the odor components between both groups and/or the presence of specific components exclusively in the odor of one group allowed the color sensor-based discrimination. The demonstrated color sensor was thus shown to be a potentially versatile and simple as an on-site screening tool. Strategies able to further improve the sensor performance were also discussed.

1. Introduction

Identification of the geographical origin of agricultural products has recently become an important issue in many countries for a fair evaluation of their commercial value and for preventing deception on the markers of origin. Since trading of agricultural products has grown enormously, it is necessary to analyze large numbers of samples from diverse geographical origins. Therefore, a simple and fast analytical method especially capable of on-site measurement is essential. Vibrational spectroscopic methods such as near-infrared (NIR) spectroscopy [1–4] have been frequently adopted as fast screening tools for this purpose. However, these methods generally require a pre-sampling step, whereby powdered samples are first obtained from raw samples, to acquire more reproducible and representative spectra. This step of drying and grinding of samples is time-consuming and particularly undesirable when quick on-site measurement is required.

Since many agricultural samples have distinct odors, direct analysis of the odors without extensive sample preparation is simple and fast. When the chemical components comprising odors from samples differ depending on the geographical origins of the samples, the compositional dissimilarity can be a versatile index for sample discrimination. The simplest analytical method for recognizing volatile aromas is an electronic nose (e-nose) developed to mimic the mammalian olfactory system [5]. It is typically composed of multiple sensor arrays and employs pattern recognition methods such as an artificial neural network (ANN) for analysis of signals from individual sensors. There are several different types of e-noses that have been described in literatures, including metal oxides [6–8], conductive polymers [9, 10], optical sensors [11], surface acoustic wave sensors [12], and electrochemical sensors

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[13]. The sensors incorporating metal-oxides and conductive polymers have been primarily used for applications such as evaluating the freshness of food [14], its ripeness [15], and shelf life [16], as well as medical pathology [17]; however, the high operation temperature and limited life of such sensors are some of the issues that still need to be addressed.

In this work, the feasibility of a M13 bacteriophage-based color sensor that can change its structural color via interaction with diverse gaseous molecules was evaluated for screening the geographical origins of three different agricultural products, namely, garlic, onion, and perilla. The M13 bacteriophage is a filamentous bacteriophage that is mainly composed of 2700 copies of helically arranged pVIII major coat proteins [18]. Typically, the size of the M13 bacteriophage is highly regular, with a length of ~880 nm and a diameter of ~6.6 nm. For fabrication of the sensor, the self-assembly of the M13 bacteriophage on the Au surface was assisted by a simple pulling technique and the regular arrangement of the M13 bacteriophage generated a characteristic structural color [19, 20]. When the color sensor was exposed to the gas molecules, the M13 bacteriophage bundles interacted with them and promptly swelled, thereby inducing a change in their structural color. The M13 bacteriophage-based color sensor has been applied for the detection of trinitrotoluene (TNT) [21], antibiotics [22], and endocrine-disrupting chemicals [23].

If the compositions of the odors emanating from agricultural samples differ depending on their geographical origin, the interactions of the odor molecules with the functional groups of amino acids on the surface of the M13 bacteriophage will be dissimilar. Therefore, the resulting color changes of the sensor can be potentially used to discriminate different geographical origins. In this study, the odor of a sample was allowed to come into contact with the color sensor in a chamber and the changes in color were continuously recorded with the increase of sample temperature by using a charge-coupled device (CCD) camera. Then, the features of acquired color (RGB) signals from each agricultural sample was examined and the RGB data was used for a support vector machine (SVM) [24] to determine the corresponding geographical origins. Finally, the discrimination accuracies were examined in relation with the corresponding RGB features.

2. Experimental

2.1. Preparation of Samples

All garlic, onion, and perilla samples used in this study were supplied by the National Agricultural Products Quality Management Service (NAQS) in Gimcheon, Republic of Korea. For each product, 20 domestic and 20 imported samples (largely from China) were included. Although the number of samples in each set was not large, the samples cultivated in diverse regions were cautiously collected by the NAQS in order to avoid duplicate sampling and to simultaneously include a wide compositional variation in the dataset. Since the odors of the samples needed to be analyzed by the color sensor, the measurement was initiated immediately on acquiring the samples.

2.2. Fabrication of M13 Bacteriophage-Based Color Sensor and Sample Measurements

The M13 bacteriophage-based color sensor was prepared by a simple pulling technique, as described previously [19, 20]. Briefly, a gold-coated Si wafer (525 μm in thickness, 100 nm of Au over a titanium adhesion layer; Platypus Technologies, Madison, WI, USA) was dipped in the Tris-buffer saline solution (12.5 mM Tris and 37.5 mM NaCl, pH 7.5) containing M13 bacteriophages with repeating units of four glutamates (concentrations: 5 mg/mL). Three different sections (Section I, II, and III) were produced on the surface by controlling the pulling speed between 10 and 100 μm/min by a programmable syringe pump (LEGATO 270; KD Scientific Co., Holliston, MA, USA); therefore, the densities of the self-assembled bacteriophage in each section on the sensor were different.

Actual odor measurements were accomplished by inserting an intended amount of the sample into a 500-mL homemade sealed plastic chamber. A razor-cut piece of garlic (1.0 g), a 1.0 g piece of onion, and 0.2 g of perilla seeds without further treatment were used for the analysis. The chamber consisted of a heating block at the bottom to control the sample temperature and the color sensor was placed on the top of the chamber fitted with a CCD microscope camera (Celestron LCC., Torrance, CA, USA) to acquire real-time RGB data. A targeting region on each color section was initially selected and the changes in the RGB signals were acquired. Using Photoshop (Adobe Systems Inc., San Jose, CA, USA), the RGB differences between the control (no sample) and the sample were calculated and visualized.

Each sample was placed on the heating block with an initial temperature setting of 30 °C. Right after sample loading, the temperature of the heating block was increased from 30 to 60 °C in 40 s (0.75 °C/s) and was maintained at 60 °C for 252 s. The temperature was again increased from 60 to 90 °C over 40 s at the same rate and was maintained at 90 °C for 252 s. During the two-step temperature increase (584 s in total), RGB data were continuously recorded at the three different sections on a color sensor. Thus, three RGB data were acquired from each sample.

2.3. Analysis of RGB Data Using SVM for Discrimination

SVM, which classifies classes using the hyperplane maximizing the margin (equivalent to the distance between classes), was employed in this study. As a kernel function for SVM, both linear and radial basis function were evaluated. In the case of using linear kernel, the degree changed from 1, 2, 3, 4, to 5 and simultaneously the cost constant (C) varied from 1, 10, 100, 1000, to 10000. Then, an optimal combination yielding a lowest discrimination error was searched. When radial basis function was used, the sigma changed from 1, 10, 100, 1000, to 10000 with the same variation of constant function for the evaluation. To run SVM, R 3.2.0 software (R Development Core Team. 2005) available from the Comprehensive R Archive Network (CRAN) at http://cran.r-project.org/ was used.

2.4. GC–MS Analysis

GC–MS (SCION TQ; Bruker Daltonics, Leipzig, Germany) equipped with a 30 m (length) × 0.25 mm (inner diameter (I.D.)) × 0.25 μm (film thickness) AB-5MS capillary column (Abel Industries Ltd., Vancouver, Canada) was used to analyze 6 randomly selected garlic and perilla samples (3 samples from each geographical origin). The sample odor was captured in a headspace with the same condition used for color sensor measurement, and 2.5 mL of captured odor were injected. The injector operated at 280 °C, and total GC running time was 36 min. The oven temperature was initially kept at 40 °C for 0.1 min. Then, the temperature was increased to 280 °C at 8 °C/min and was kept constant for 5 min. Helium (99.9990%) was used as a carrier gas. The scan range was set from 45 to 400 m/z.

3. Results and Discussion

In this study, the ΔRGB intensity corresponding to the change in each color relative to the control acquired without the exposure of sample odor was evaluated. Fig. 1(a) shows the variation in the average ΔRGB intensities on each section as the temperature increased in the measurement of garlic samples. The color sensor incorporating M13 bacteriophages with repeating units of four glutamates (4E color sensor) on the surface was used as described. The solid and dotted lines correspond to the domestic and imported garlic samples, respectively. In each case, 60 acquired ΔRGB intensities (20 samples, triplicate measurements per sample) were averaged. Since the three sections had

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