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Discrimination and growth tracking of fungi contamination in peaches using electronic nose

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ABSTRACT

A non-destructive method for detection of fungal contamination in peaches using an electronic nose (E-nose) is presented. Peaches were inoculated with three common spoilage fungi, *Botrytis cinerea*, *Monilinia fructicola* and *Rhizopus stolonifer* and then stored for various periods. E-nose was then used to analyze volatile compounds generated in the fungi-inoculated peaches, which was then compared with the growth data (colony counts) of the fungi. The results showed that changes in volatile compounds in fungi-inoculated peaches were correlated with total amounts and species of fungi. Terpenes and aromatic compounds were the main contributors to E-nose responses. While principle component analysis (PC1) scores were highly correlated with fungal colony counts, Partial Least Squares Regression (PLSR) could effectively be used to predict fungal colony counts in peach samples. The results also showed that the E-nose had high discrimination accuracy, demonstrating the potential use of E-nose to discriminate among fungal contamination in peaches.

1. Introduction

Peach (*Prunus persica* (L.) Batsch) is one of the most favorable fruits in the market owing to its high nutrient content and pleasant flavor. However, peach can easily be contaminated with pathogenic spoilage fungi during harvest, processing, and transportation. The major post-harvest fungi in peaches that are responsible for limited storage and shelf life include *Botrytis cinerea*, *Monilinia fructicola* and *Rhizopus stolonifer*, which can cause the gray mold rot, brown rot and *Rhizopus* soft rot of peaches, respectively (Casals, Teixidó, Viñas, Cambray, & Usall, 2010; Karabulut et al., 2002; Zhang, Zheng & Yu, 2007). Several methods have been developed to monitor fungi contamination in peaches, and these include microbiological cell counting, thin layer chromatography (Overy, Seifert, Savard, & Frisvad, 2003), high-performance liquid chromatography (León-Zapata et al., 2016), and enzyme-linked immunosorbent assay (Thornton, Slaughter & Davis, 2010). Although these methods are precise and effective, they are time-consuming, and operationally complicated and destructive. Moreover, they cannot be used in online detection. Hence, it is important to develop the methods that are fast, sensitive, and non-destructive to detect and monitor the contaminated fungi in peaches.

In recent years, a series of non-destructive methods for the detection of microbes have been developed. These methods include near-infrared (Moscetti et al., 2015), computer version (Pan et al., 2017), hyperspectral imaging (Sun et al., 2015), and mid-infrared spectroscopy (Kaya-Celiker, Mallikarjunan & Kaaya, 2015). However, the spectroscopic methods generally require considerable amount of spectral pretreatment prior to establishing the complex models (Xu, Yu, Liu, & Zhang, 2016). Moreover, small changes between fungi contamination and surface characteristic in sample may limit their ability to monitor the contamination. Unlike the methods mentioned above, electronic nose (E-nose) is a solid-state sensor-based system, consisting of the data collection unit and the computerized statistical data processing tool. The system relies on a principle of which different volatile chemical compounds contribute to the characteristic flavor/aroma of the samples. The metal oxide sensors of the E-nose can collect volatile compound data and give outputs as the so-called 'fingerprints' to represent the characteristic flavor/aroma (Hartyáni et al., 2013). Undesired smells in food, which primarily come from microbial metabolism (Sanaeifar, Zakidizaji, Jafari, & Guardia, 2017) can be detected by E-nose. Previous studies have demonstrated that E-nose technology can be used for the assessments of fruit ripening, firmness, sugar content, and pH values (Rizzolo et al., 2013; Zhang, Wang, Ye, & Chang, 2012). Other studies have proved that E-nose can detect early contaminations and defects caused by microorganisms in strawberry (Pan, Zhang, Zhu, Mao, & Tu, 2014), blueberry (Li, Krewer, Ji, Scherm, & Kays, 2010), orange (Pallottino et al., 2012), tomato, and fruit juice (Thornton et al., 2010). However, these researches were stalled at the preliminary qualitative discrimination for the samples. The feasibility of using E-nose to quantitatively detect individual fungal contamination as well as to

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monitor their growth in peaches has not been adequately evaluated.

Therefore, we developed the discriminative and quantitative models of pathogenic fungi contamination in peaches as follows: (1) we collected and investigated the sensor responses of three common fungal species using E-nose; (2) we confirmed and validated the correlations between the sensor responses and the volatile compounds using gas chromatography-mass spectrometry; (3) we developed models for monitoring the growth of fungi in peaches during storage period; and (4) we evaluated the feasibility of E-nose in discriminating among the inoculated fungi.

2. Materials and methods

2.1. Sample preparation

Peach samples (*Prunus persica* (L.) Batsch var. Xia 8) were purchased from Jiangsu Academy of Agricultural Sciences in Nanjing of Jiangsu province, China on August 1st, 2017. Peaches were selected based on their ripeness (80%) and shape, and were visually inspected for the absences of bruised surface and fungal contamination. Immediately after the selection process at the orchard was completed, the peaches were transported to the laboratory. The samples were immersed in 0.1% (V/V) trichloroacetic acid for 2 min, and then rinsed twice with sterile distilled water. After that, they were randomly divided into four groups.

Three major postharvest pathogenic fungi, *Botrytis cinerea (B. cinerea*, isolated from the surfaces of infected strawberry, identification number: ATCC 58025), *Monilinia fructicola (M. fructicola* isolated from the infected honey peach, identification number: ATCC 44557), and *Rhizopus stolonifer (R. stolonifer*, isolated form the infected peach, identification number: ATCC 24862), were provided by the Guangdong Microbiology Culture Center (Guangzhou, China), and used for inoculation. The fungi strains were grown on potato dextrose agar medium (PDA) at a 27 °C in an 85% relative humidity atmosphere for 1 week prior to inoculation. After each fungus spore type was re-suspended from PDA surface, its concentration was measured using a hemocytometer (Sun et al., 2015), and adjusted to final concentration of 1×10^5 spores mL^{-1} with sterile saline solution (0.85% (V/V) NaCl).

For fungal infection, peach samples were inoculated with 20 μL of suspension containing spore of *B. cinerea*, *M. fructicola* or *R. stolonifer* concentration at a 2-mm depth. Peaches inoculated with sterile saline solution were used as controls (CK group). Following moisture was allowed to evaporate at room temperature (25 ± 3 °C), the samples were placed in polyethylene plastic boxes and incubated at 20 °C under 85% relative humidity conditions. In order to obtain the growth situation of *B. cinerea, M. fructicola* and *R. stolonifer*, five peaches from each group were subjected to microbiological analysis at various time points (i.e. 0, 12, 24, 36, 48, 60 and 84 h). Because growths of *M. fructicola* and *B. cinerea* were slower than that of *R. stolonifer* based on our per-experiment and previous work (Sun et al., 2015), samples inoculated with these two fungi were also examined at 96 and 108 h in order to obtain their complete growth profiles. The total number of peaches inoculated with *B. cinerea*, *M. fructicola*, and *R. stolonifer* were 50, 45, and 40, respectively, and that of control was 50. At each time point, a total of 20 peach samples (five from each group) were randomly sampled for E-nose detection and then subjected to microbiological analysis. The simplified step-by-step experimental procedures are illustrated in Fig. S1a (supplementary material).

2.2. E-nose system

A commercial portable E-nose system (PEN3, Win Muster Air-sense Analytics Inc., Germany) was used to acquire the aromatic information (i.e., volatile compounds data). The E-nose system consists of three units: the sampling unit, the gas detection system, and the pattern recognition software. In addition, the gas detector of the E-nose system is composed of 10 metal oxide sensors (MOS), which are differentially sensitive to each characteristic volatile compound. The general description of each sensor is shown in Table S1. In the experiment, gas was injected via probes into the detection system from the headspace of the samples at a constant rate. The response value (G/G_0) of each sensor changed accordingly to the composition of volatile compounds [G and G_0 stand for the conductivity of the MOS connected with the sample and clean gas, respectively (Pan et al., 2014)].

For the measurements, the peach sample was placed in a 250 mL beaker, which was then sealed with a (breakable) silver paper (at the top). After that, the headspace of the sample was equilibrated for 10 min at a constant temperature (25 °C) to minimize sensor drift due to environmental changes (Baldwin et al., 2011). The gas headspace was pumped over the sensor surfaces for 60 s at a constant flow rate of 150 mL min^{-1}, which was long enough for the sensors to reach stable signal responses (Fig S1b). Following the probe was cleaned with filtered air for 120 s, the baseline was set (by auto-zero) for 5 s prior to the following measurement. In this work, the stable value of each sensor of measurement were extracted and used for further data analyzing. Each sample was analyzed in triplicate, and the mean values were used for data processing. The five replicates (samples) were completed for each group (CK, *B. cinerea, M. fructicola* and *R. stolonifer* group). Fisher's least significant differences (LSD) test was used for investigation of statistically significant differences ($P < 0.05$) in mean values of each sensor (Granato, Calado, & Jarvis, 2014).

2.3. Analysis of volatile organic compounds

Volatile compounds from the peach samples were identified and analyzed using a headspace solid phase micro extraction in conjunction with gas chromatography/mass spectrometry (HPSE-GC/MS). The compounds were extracted and concentrated using a PDMS/DVB fiber (65 μm; Supelco, PA, USA). They were then separated and identified by an Agilent 7890 GC/MS (Agilent Technologies Inc., CA., USA). The HPME-GC/MS analysis procedure has been detailed in Wang et al. (2009), and was carried out with some modifications.

For each headspace extraction, 5.0 ± 0.3 g of the peach sample was ground into powder in liquid nitrogen. Zero point six gram of NaCl and 10 μL of 3-octanol (0.01 mg mL^{-1}; an internal standard) were then added. The mixture was added into a 20 mL vial and then sealed with silicone rubber mat. The vial was equilibrated at 45 °C in a water bath. The PDMS/DVB fiber was exposed to the headspace of the sample to allow absorption of the volatile organic compounds for 30 min. After that, the fiber was injected into GC for desorption for 5 min at 250 °C. The column temperature was initially set to 40 °C and was then increased to 150 °C at a constant ramp up rate of 2 °C min^{-1}. Subsequently, the temperature was increased to 210 °C at a constant ramp up rate of 10 °C min^{-1} and was kept constant for 2 min to allow re-equilibration. The MS was operated in the electron impact ionization mode (70 eV) and the data were scanned from 30 to 450 *m/z*. Temperatures of ion source and quadrupole were 230 °C and 150 °C, respectively. The data for each group were from replicate experiments and were presented as μg g^{-1} FW equivalent of 3-octanol.

2.4. Colony count

After E-nose acquisition, the total colony count was carried out according to the Chinese official analysis method (Chinese Official Document number: GB 4789.15-2016). Briefly, 25 g of sample was mixed with 225 mL sterile saline solution for 2 min. After that, 1 mL of the colony suspension was enumerated on Rose Bengal medium for 4 days at 27 °C and 85% relative humidity. The colony was counted as colony-forming unit and was presented as log (CFU g^{-1}) (This common log is based 10). Each sample was analyzed in triplication, and the mean values were used for data processing.