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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.

2.5. Data analysis

In this work, growths of the three fungal species from peach samples were described according to the following mathematical model (Logistic model) (Gibson & Hocking, 1997):

$$N = N_0 + \frac{(N_{max} - N_0)}{1 + e^{(\mu_{max}*(\lambda - t))}}$$

where N_0 and N_{max} (unit: log (CFU g^{-1})) are the initial and the maximum colony count, respectively; μ_{max} (unit: h^{-1}) is the maximum fungal growth rate; λ (unit: h) is the lag time of the fungal growth; t (unit: h) is the storage time. The adjusted correlation coefficients (adj. R^2) were used to evaluate the performance of the growth models based on colony count.

Principal Component Analysis (PCA) was applied to reduce the dimensions of the E-nose data, by which the only key feature data were used. The details for PCA theoretical definition were introduced in previous work (Grané and Jach, 2014). The sensor response value obtained from E-nose was pre-treated with Standard Normal Variate (SNV) to eliminate the signal drift. Partial Least Squares Discrimination Analysis (PLS-DA) was adopted to build the classification models for individual fungal species, and Partial Least Squares Regression (PLSR) analysis was utilized to develop the determination model for colony counts. Data from the three fungi-inoculated groups used in the prediction of colony counts were divided into two sets: calibration set and prediction set at a ratio of 2:1, according to Kennard Stone algorithms (calibration set: prediction set = 33:17, 30:15, and 27:13 for *B. cinerea, M. fructicola,* and *R. stolonifer* groups, respectively) (Gu, Sun, Kang, Dong, & Pan, 2016; Pan et al., 2017). Various parameters, including correlation coefficient of determination (R^2), root mean square error of calibration (RMSEC), root mean square error of prediction (RMSEP), and residual predictive deviation (RPD), were used to evaluate the performance of the models. The parameters were defined as follows:

$$R_C^2,\ R_P^2 = 1 - \frac{\sum_{i=1}^{n} (y_i - \bar{y}_i)^2}{\sum_{i=1}^{n} (y_i - y_m)^2}$$

$$\text{REMSEC, RMSEP} = \sqrt{\frac{1}{n} \sum_{I=1}^{n} (y_i - \bar{y}_i)^2}$$

$$\text{RPD} = \frac{\sqrt{\frac{1}{n-1} \sum_{i=1}^{n} (y_i - y_m)^2}}{\sqrt{\frac{1}{n} \sum_{i=1}^{n} (y_i - \bar{y}_i)^2}}$$

where n is the total number of samples; y_i and $\bar{y}$ are the measured and the predicted values of the i-th sample, and y_m is the mean value of all samples in calibration or prediction set. As has been described in Chang (2001), a model that has an RPD value of greater than 2.0 is considered having excellent performance.

One-way analysis of variance (ANOVA) was used to test the significance (LSD, $P < 0.05$) of mean difference among different groups of samples (expressed as means ± standard deviation (SD)). The SNV processing as well as the classification and determination models were built with PLS toolbox 7.5 (Eigenvector Research, Inc., Wenatchee, WA, USA), an integrated software in Matlab 7.9 (MathWork Inc., Natick, MA, USA). Additionally, PCA was performed using SPSS 18.0 software (IBM Corporation, Armonk, NY, USA), and the graphs were prepared with Origin 9.0 (Origin Lab Corporation, Northampton, MA, USA).

3. Results and discussion

3.1. Growths of B. cinerea, M. fructicola, and R. solonifer in peach samples during storage

The total counts of *B. cinerea*, *M. fructicola* and *R. stolonifer* in peach samples determined using the Chinese official analysis method. Colony counts for *B. cinerea*, obtained from 0 to 108 h, were 1.60, 1.66, 2.00, 2.21, 2.68, 3.06, 3.62, 3.92, 4.24 and 4.31 log (CFU g^{-1}), as shown in Fig. S2 & Table S2. While colony counts for *M. fructicola* (obtained from 0 to 96 h) were 1.66, 1.85, 2.06, 2.67, 2.93, 3.78, 4.73, 4.99 and 5.01 log (CFU g^{-1}), those for *R. stolonifer* (obtained from 0 to 84 h) were 1.88, 2.01, 2.06, 3.12, 3.79, 4.45, 5.15 and 5.34 log (CFU g^{-1}). In the first 24 h of the storage, the average value of total colony counts changed slowly, but the significant difference ($P < 0.05$) was observed between CK group and fungal-infected groups. From the 24 h to 72 h period, the colony counts for *B. cinerea*, *M. fructicola* and *R. stolonifer* increased with 1.62, 2.72 and 3.09 log (CFU g^{-1}), respectively. Rising trend for *R. stolonifer* group tends to be stable for the 84 h, but the *B. cinerea* group was still with a constant increasing. Therefore, this variation tendency of total colony count for each group could reflect the characteristic of postharvest pathogenic fungi.

The growth-fitting results obtained from the growth simulation for *B. cinerea*, *M. fructicola* and *R. stolonifer* in peaches (Fig. S2), and the detailed equations and parameters of the growth curves for each fungal group are tabulated in Table S3. High adj. R^2 were obtained from the growth-fitting models with values of 0.997, 0.983, and 0.985 for *B. cinerea*, *M. fructicola,* and *R. stolonifer*, respectively. According to the total colony counts, while growths of all three fungal species were not explicit in the first 24 h, those of *R. stolonifer* and *M. fructicola* increased more sharply than those of *B. cinerea* after 24 h. It is known that fungi growth is divided into three phases: the lag phase, the exponential phase, and the stationary phase. According to the coefficients obtained from the growth curves, *B. cinerea* had the longest lag phase (56.708 h) and the slowest growth rate (0.051 h). The results demonstrate that *R. stolonifer* in the peach samples stored at the experimental condition has better reproduction ability than *M. fructicola* and *B. cinerea*.

3.2. Volatile compounds data in four groups of peaches from HPSE-GC/MS

Following HPSE, 37 volatile compounds in the four groups of peaches were identified and qualified by GC/MS (Table 1). These compounds were identified to be seven aldehydes, eleven esters, nine alcohols, four lactones, one acid, and five olefins. Among these, hexanal, E-2-hexenal, E-2-nonenal, benzaldehyde, hexyl acetate, alcohols, γ-octalactone, and γ-decalactone have been observed to be the major characteristic volatile components of non-infected peach (Engel et al., 1988; Hu, Wang & Li, 2007; Wang et al., 2009). In the present study, eight compounds, including hexanal, E-2-hexenal, hexyl acetate, Z-2-hexen-1-ol, linalool, γ-octalactone, γ-decalactone, and β-myrcene were found to be the major volatile compounds contributing to the characteristic flavor of peaches in CK group (control). Similar observation has been reported by Horvat et al. (1992), where hexanal, E-2-hexenal, benzaldehyde, linalool, γ-octalactone and γ-decalactone were demonstrated to be the six major characteristic volatile compounds of peach-like aroma.

Comparing CK group with the fungi-infected group showed that the eight major volatile compounds were significantly different ($P < 0.05$). Several compounds (e.g., hexyl acetate) were not detected in fungi-infected groups after 72 h of storage. Although seven aldehydes were detected in all four groups, hexanal, E-2-hexenal, and benzaldehyde were the only commonly detected compounds during the entire storage period. Other compounds including furfural, E-2-eptenal, and 2-dodecenal were detected only in *R. stolonifer* group at 72 h. At the early storage period, three esters were found in CK group. At 72-h storage period, seven, five, and eight esters were detected in peaches infected

Table 1
Major volatile compounds detected and quantified in peaches samples of four groups by HPSE-GC–MS during the storage (Unit: μg g^{-1} FW equivalent of 3-octanol).

Category	Volatile Compound	CK			Fungal species					
					B. cinerea		*M. fructicola*		*R. stolonifer*	
		0 h	48 h	72 h	48 h	72 h	48 h	72 h	48 h	72 h
Aldehydes	hexanal	77.87 ± 19.83b	149.18 ± 28.01c	137.28 ± 8.96c	98.04 ± 9.66bc	76.41 ± 12.56b	157.69 ± 8.26c	59.46 ± 30.40b	114.28 ± 15.82c	10.07 ± 0.17a
	E-2-hexenal	115.83 ± 32.37bc	98.95 ± 17.67b	109.33 ± 12.46b	118.84 ± 11.08b	47.62 ± 22.33a	145.55 ± 6.75c	42.93 ± 9.88a	135.84 ± 16.2c	18.12 ± 2.02a
	furfural	\	\	\	\	\	\	\	\	18.78 ± 5.07
	benzaldehyde	56.35 ± 14.14a	38.79 ± 7.60a	44.07 ± 2.64a	81.78 ± 9.93b	70.84 ± 5.72b	89.17 ± 0.69c	58.87 ± 1.57a	88.44 ± 21.76c	59.66 ± 1.85a
	E-2-nonenal	\	\	\	\	\	\	\	\	24.32 ± 4.54
	Z-2-heptenal	1.07 ± 0.05a	2.32 ± 0.69a	1.79 ± 1.29a	11.84 ± 10.81ab	\	4.37 ± 0.28a	2.61 ± 0.58a	5.80 ± 0.88b	
	2-dodecenal	\	\	\	\	\	\	\	\	5.62 ± 1.61
Esters	heptyl acetate	\	\	\	\	2.46 ± 3.48a	\	20.86 ± 1.94a	\	\
	hexyl acetate	91.97 ± 3.43a	124.14 ± 27.14b	116.44 ± 23.07b	110.15 ± 16.51b	\	127.85 ± 19.73b	\	147.31 ± 14.16b	\
	methyl benzoate	\	\	\	\	4.01 ± 3.40a	\	\	\	3.32 ± 1.75a
	ethyl benzoate	\	\	\	\	58.53 ± 27.81b	\	57.50 ± 20.91b	10.48 ± 1.31a	41.88 ± 7.69b
	ethyl caprylate	\	21.66 ± 5.63c	23.04 ± 0.10c	36.59 ± 14.65c	9.03 ± 2.79b	11.78 ± 1.90b	5.80 ± 1.01a	9.62 ± 1.06b	4.39 ± 0.50a
	propanoic acid, 2-octyl ester	1.51 ± 0.13a	\	3.45 ± 0.24ab	2.39 ± 0.38b	8.40 ± 4.23c	4.00 ± 2.83ab	5.17 ± 1.07bc	5.66 ± 0.69bc	5.12 ± 1.04bc
	citronellyl acetate	\	\	\	\	\	\	\	\	8.14 ± 1.23
	acetic acid, non-3-enyl ester	\	\	\	\	7.46 ± 1.70b	\	2.85 ± 0.43a	2.50 ± 0.37a	4.80 ± 0.21a
	bromoacetic acid, dodecyl ester	\	\	\	\	\	\	\	\	2.12 ± 1.90
	benzyl acetate	0.41 ± 0.04a	1.35 ± 0.79b	1.27 ± 0.05b	3.37 ± 1.11c	2.52 ± 0.71c	2.32 ± 0.08c	\	1.33 ± 0.39b	\
	phthalic acid, decyl isobutyl ester	\	\	\	\	\	\	\	\	0.32 ± 0.05
Alcohols	4-ethyl-1-hexyn-3-ol	0.10 ± 0.04a	\	\	\	\	4.40 ± 0.70b	\	0.97 ± 0.78a	\
	2-furanmethanol	\	\	\	9.78 ± 2.37ab	\	\	\	\	\
	Z-2-hexen-1-ol	23.34 ± 13.35b	54.16 ± 12.03 cd	50.34 ± 1.11c	66.77 ± 10.41de	\	117.51 ± 7.43e	1.71 ± 1.45a	91.79 ± 8.86 h	0.70 ± 0.21a
	E-2-hexen-1-ol	12.00 ± 3.07c	5.62 ± 0.64b	9.84 ± 3.91a	\	1.39 ± 1.02a	\	\	\	\
	linalool	147.30 ± 10.14b	204.49 ± 39.81c	216.78 ± 12.42c	215.96 ± 31.29c	135.59 ± 65.45b	296.30 ± 21.19d	145.63 ± 23.65b	215.05 ± 18.63c	26.13 ± 9.93a
	geraniol	12.09 ± 2.28b	\	2.36 ± 1.34a	\	6.08 ± 4.34a	\	\	\	\
	4-carvomenthenol	\	7.25 ± 1.47a	6.84 ± 1.87a	6.98 ± 0.53a	6.48 ± 4.96a	7.64 ± 0.11a	\	\	\
	β-citronellol	\	\	\	\	\	\	\	\	9.52 ± 6.49
	3-methyl-3-cyclohexen-1-ol	\	2.26 ± 0.16b	0.78 ± 0.06a	1.81 ± 0.63b	0.86 ± 0.61a	\	\	1.67 ± 0.53ab	\
Lactones	camphor	1.24 ± 0.22a	9.36 ± 0.63c	4.19 ± 1.04b	10.23 ± 1.57c	11.07 ± 4.06c	10.54 ± 0.78c	11.39 ± 2.60c	10.74 ± 3.57c	4.63 ± 1.31b
	γ-octalactone	7.24 ± 2.50c	8.98 ± 0.52c	9.77 ± 0.19c	7.62 ± 2.54c	2.61 ± 0.16a	6.36 ± 4.46b	1.46 ± 0.29a	1.34 ± 0.38a	0.63 ± 0.30a
	γ-decalactone	8.99 ± 0.08d	10.38 ± 1.50d	13.84 ± 1.10e	4.05 ± 1.07b	4.63 ± 2.48b	5.20 ± 1.37c	1.05 ± 0.23a	5.99 ± 0.80c	2.46 ± 1.88a
	3-ethylcyclopent-2-enone	\	\	\	4.02 ± 3.53b	1.13 ± 1.11a	3.83 ± 1.49b	\	1.82 ± 0.42ab	0.50 ± 0.11a
Acids	acetic acid	6.35 ± 2.95a	5.46 ± 1.57a	6.12 ± 2.34a	3.69 ± 0.06a	\	2.63 ± 0.46a	\	6.49 ± 1.38a	\
Olefins	2-ethyl-3-vinyloxirane	2.67 ± 1.94b	\	0.71 ± 1.00a	1.14 ± 1.62ab	\	\	\	\	\
	β-myrcene	9.81 ± 1.79a	14.53 ± 3.41a	12.90 ± 0.67a	15.80 ± 4.22b	26.24 ± 5.58c	18.09 ± 1.21b	17.90 ± 2.81b	18.29 ± 2.64b	16.42 ± 2.10ab
	α-pinene	\	1.05 ± 1.00a	\	4.80 ± 3.57b	4.20 ± 0.62b	7.04 ± 1.25b	5.73 ± 0.57b	3.70 ± 2.95a	2.52 ± 0.41a
	tran-decalin	\	0.42 ± 0.31a	0.72 ± 0.23a	2.62 ± 0.89b	2.08 ± 0.54b	\	1.59 ± 0.42a	\	2.03 ± 0.70b
	hexadecane	\	1.58 ± 1.48a	2.78 ± 0.90b	3.08 ± 0.06b	2.54 ± 0.92a	6.57 ± 2.06c	0.85 ± 0.66a	5.41 ± 1.20c	\

'\' = not detected; Values with different letter in the same row are significantly different ($P < 0.05$).

with *B. cinerea*, *M. fructicola*, and *R. stolonifer*, respectively. It is worth noting that 72 h following the fungi infection, the content of hexyl acetate in all infected groups was sharply decreased (116.44 μg g^{-1} FW), while that of ethyl benzoate was increased to between 7.50 and 58.53 μg g^{-1} FW, suggesting that ethyl benzoate may contribute to overall aroma of samples. The contents of alcohol compounds, Z-2-hexen-1-ol and linalool, were remarkably different (greater than 48.63 and 71.15 μg g^{-1} FW) between the CK and the fungi-infected groups. Lactones have been reported as the typical 'peach-aroma' compounds in peaches, especially for γ-decalactone (Wang et al., 2009). The content of γ-octalactone in the fungi-infected peaches significantly decreased ($P < 0.05$), from 7.24 to 0.63–2.61 μg g^{-1} FW. Similarly, the content of γ-decalactone also significantly decreased ($P < 0.05$), from 8.99 to 1.05–4.63 μg g^{-1} FW in fungi-infected peaches. In contrast, the contents of camphor and 3-ethylcyclopent-2-enone in fungi-infected peaches increased from 3.39 to 10.15 μg g^{-1} FW and from 0 to 1.13 μg g^{-1} FW, respectively. These may be due to that the releasing characteristic of aroma is gradually weakened over the storage period (Ortiz, Graell, López, Echeverría, & Lara, 2010; Rizzolo et al., 2013). Moreover, among the five olefins, the differences in the amounts of β-myrcene and α-pinene between the CK and the fungi-inoculated groups were most remarkable. These findings demonstrate that the fungi infection in peaches can result in significant deterioration of the characteristic peach aroma.

The compositions of volatile compounds in peach samples inoculated with three fungi species were different. While furfural, E-2-nonenal, 2-dodecenal, citronellyl acetate, and β-citronellol were detected only in peaches infected with *R. stolonifer*, heptyl acetate was found only in those infected with *B. cinerea* and *M. fructicola*. The contents of hexanal, E-2-hexenal, and linalool in the group infected with *R. stolonifer* were significantly lower ($P < 0.05$) than those of the group infected with *B. cinerea* or *M. fructicola*. Additionally, the contents of benzaldehyde, heptyl acetate, ethyl caprylate, and hexadecane in peaches infected with *B. cinerea* were significantly higher ($P < 0.05$) than those of peaches infected with *M. fructicola* or *R. stolonifer*. Therefore, these compounds or their contents may be used as the markers determining specific fungi species. These results indicate that the decreases or absence of the characteristic peach aroma compounds as a result of fungi infection can be beneficial to the identification of fungal contamination as well as the discrimination of fungal species. Moreover, the contents of these major volatile components, which had varied detectable ranges (e.g., the detection limit of No. 9 sensor in PEN3 system for aromatic compounds was 100 ppm), could be used as the key parameters in MOS discrimination between the four groups examined in this work (CK, *B. cinerea*, *M. fructicola*, and *R. stolonifer*).

3.3. E-nose response signal of volatile compounds in peaches

The signals of 10 sensors in response to volatile compounds from different groups of peach samples over different storage periods are presented in Fig. 1. The response curve represents the G/G_0 value of the sensor as a function of storage time. The responses of all sensors to fungi-inoculated peaches significantly varied over the storage period (except for No. 10 and No. 4 sensors). These sensor responses were also highly related to the colony counts. In CK group, the G/G_0 values of all sensors changed slightly; the changes were not higher than 0.50. In contrast, while the G/G_0 values for several sensors were dramatically varied in all infected groups (*B. cinerea*, *M. fructicola*, and *R. stolonifer*), the variations were different. Sensors No. 7 and No. 9 were most sensitive to the volatile compounds from the infected peaches. The signals of sensors No. 6, No. 8, and No. 2 were not significantly changed compared with those of sensors No. 7 and No. 9. The signals of sensors

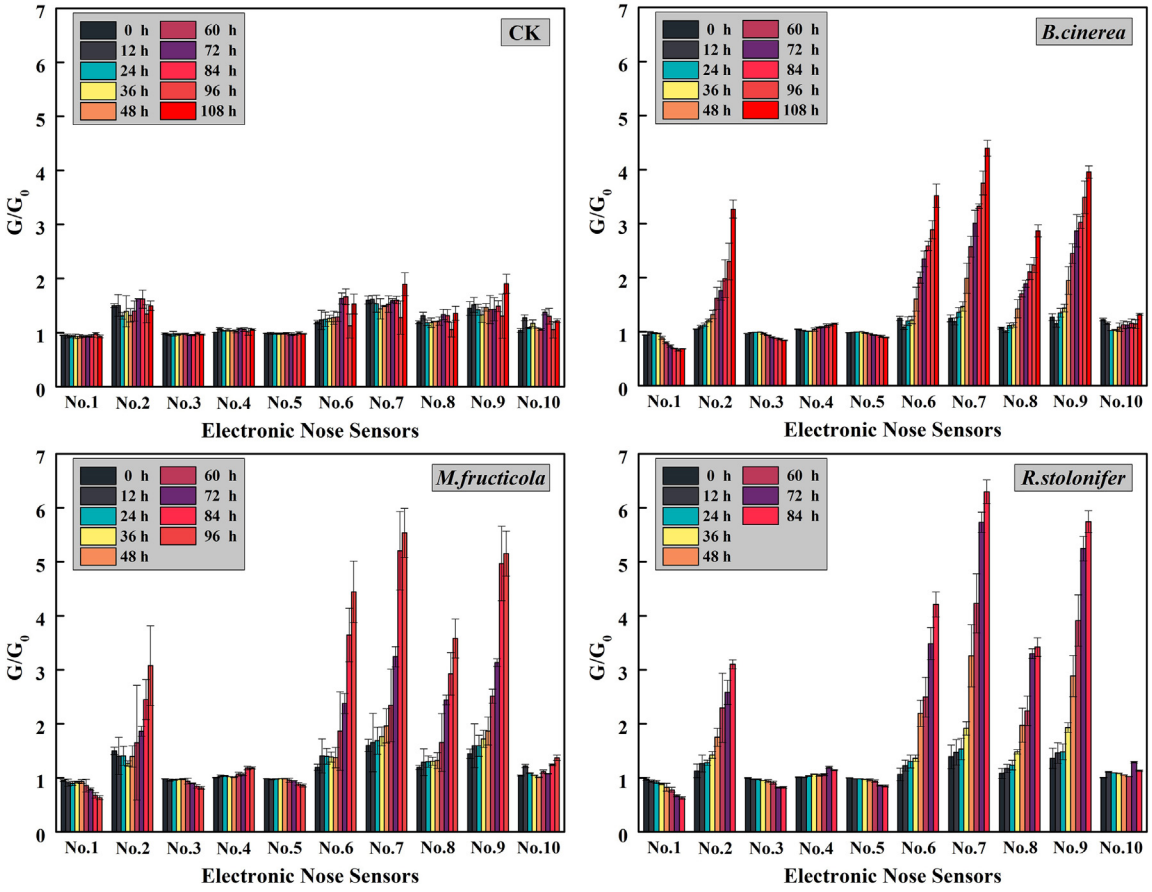

Fig. 1. Response values of ten sensors to volatile compounds from different groups of peach samples over different storage periods.

No. 10 and No. 4 in response to volatile compounds from all groups remained almost constant for the entire storage period.

Furthermore, as shown in Fig. 1, the responses of the same sensors were largely diverse among the three different fungi-infected groups. For instance, the slope of the response curve of sensor No. 8 was statistically different ($P < 0.05$) among the three infected groups. The G/G_0 reached a value of 3 at 84, 60, and 48 h for *B. cinerea*, *M. fructicola*, and *R. stolonifer*, respectively. Moreover, at the end of the storage period (84 h), the G/G_0 value for *R. stolonifer* group (5.70) was higher than that for *M. fructicola* (5.2) and *B. cinerea* (3.3) groups. The results demonstrated that the changes of volatile compounds in infected peaches were varied according to fungi counts and species, indicating that E-nose is able to discriminate among different peaches' contaminated fungal species.

High degree of multicollinearity commonly exists in E-nose sensor responses, and making it hard to precisely evaluate the dependent variables (Pan et al., 2014). Correlation coefficients for the E-nose responses and colony counts is shown in Table S4. It is obviously that colony counts significantly correlated with the No. 7 and No. 9 sensors. Apart from the No. 10 sensor, other six sensors were highly correlated with colony counts at $P < 0.05$ level. Similar results for multicollinearity among the E-nose sensors were also reported by Qiu and Wang (2017). To reduce the redundancy among the sensors, the PCA was then chosen in this work for data processing.

3.4. Correlations between sensor responses and volatile compounds

The analysis of GC/MS data showed that different compositions and contents of volatile compounds among all four groups of samples resulted in different observed responses to the 10 sensors. The responses were mainly contributed by olefins, aromatic, and aldehyde compounds. According to the discussion above and the data shown in Fig. 1, the E-nose sensors No. 7 and No. 9 were most sensitive, as indicated by their highest responses to fungi-infected peach samples. The properties of selected E-nose sensors were linked to sulfur compounds, terpenes, aromatic compounds, nitrogen oxides, and other broad range of compounds; with a sensitivity of 1 ppm (Table. S1). Although certain compounds, such as nitrogen oxides and sulfur compounds were not detected by GC/MS, several terpenes, such as β-myrcene and α-pinene, were detected in the fungi-inoculated groups. The total counts of terpenes in the infected groups were significantly higher ($P < 0.05$) than that in the CK group, and their concentrations also gradually increased over the storage period. Because sensor No. 7 is sensitive to many organic sulfur-containing compounds and terpenes, it was thus able to discriminate between the CK group and the fungi-inoculated groups. In addition, the overall concentration of aromatic compounds (benzaldehyde, methyl benzoate, ethyl benzoate, and benzyl acetate) were clearly increased (at 72 h after fungi inoculation), from 56.76 to 135.93, 116.37, and 104.86 μg g^{-1} FW in *B. cinerea*, *M. fructicola*, and *R. stolonifer* groups, respectively (Table 3). On the other hand, such concentration in the CK group was decreased from 56.76 to 45.34 μg g^{-1} FW. The observations were also corresponded with those of the sensor No. 9, which is sensitive to aromatic compounds at low concentration (1 ppm). Other sensors, such as No. 2, No. 6, and No. 8 sensors, also showed relatively high responses to fungi-infected peaches after 72 h post-infection. The sensor responses were closely linked with quality of peach (e.g., firmness, and compositions of sugar and acid) and their shelf life (Benedetti, Buratti, Spinardi, Mannino, & Mignani, 2008; Brezmes et al., 2000; Rizzolo et al., 2013). Previous studies (Xiao, 2010; Zhang, 2010) have demonstrated that *M. fructicola* and *R. stolonifer* were able to not only accelerate the decomposition of soluble solids, titratable acid, and vitamin C, and the deterioration of fruit firmness, but also increase the contents of ethylene. These broad range metabolites have been reported to be highly and positively related to the sensors No. 2, No. 6, and No. 8 (Benedetti et al., 2008; Brezmes et al., 2000; Rizzolo et al., 2013). The observations in the present work also showed that other volatile organic compounds, such as E-2-nonenal, furfural and citronellyl acetate, were differentially produced and released in relation to the three fungal species. Thus, these volatile compounds could be used to discriminate among the fungal species.

3.5. Analysis of the E-nose response by PCA

In order to extract the main features, reducing the dimensions of the original variables, PCA was applied to analyze the E-nose data, by which main information was retained in several unrelated principal components (Gu et al., 2016). In general, when the accumulated contribution of certain principal components (PCs) is over 85%, the PCs can represent the original data. The data showing the loading analysis of E-nose response of infected peaches at different storage periods is shown in Fig. S3. The first two principal components, which were

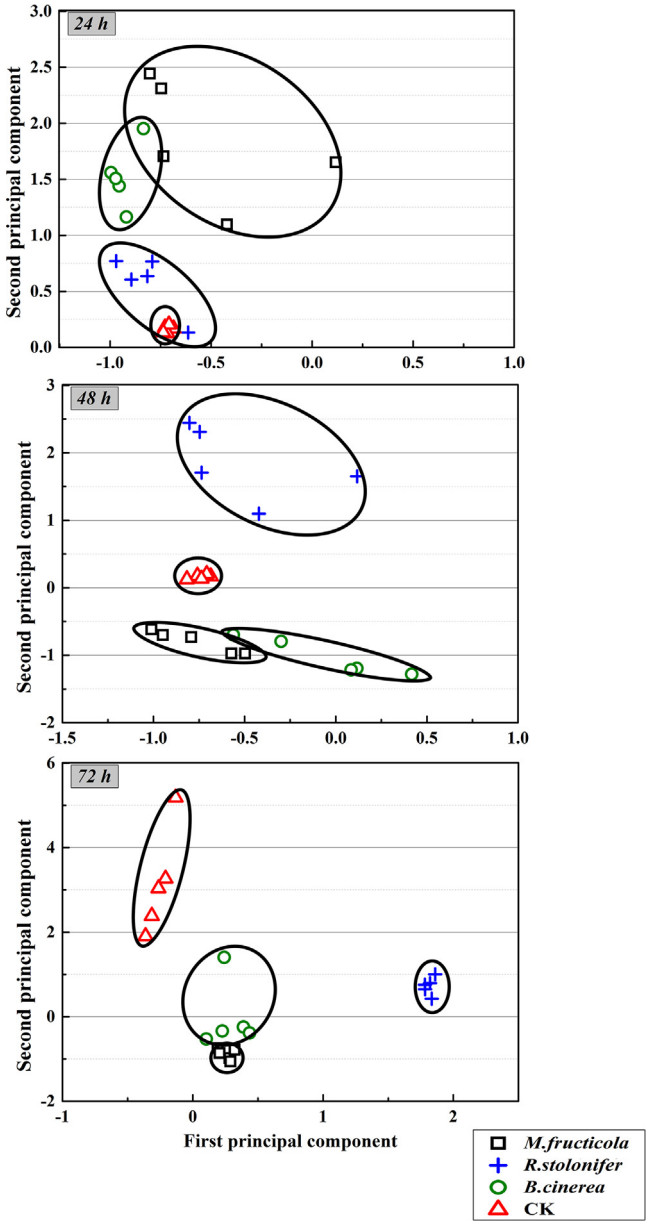

Fig. 2. Comparison of loading analysis of E-nose data in response to volatile compounds generated from peach samples inoculated with *B. cinerea, M. fructicola*, and *R. stolonifer* at different storage periods. ○ means *B. cinerea*-inoculated group; □ means *M. fructicola*-inoculated group; + means *R. stolonifer-inoculated* group; △ means control group, respectively.

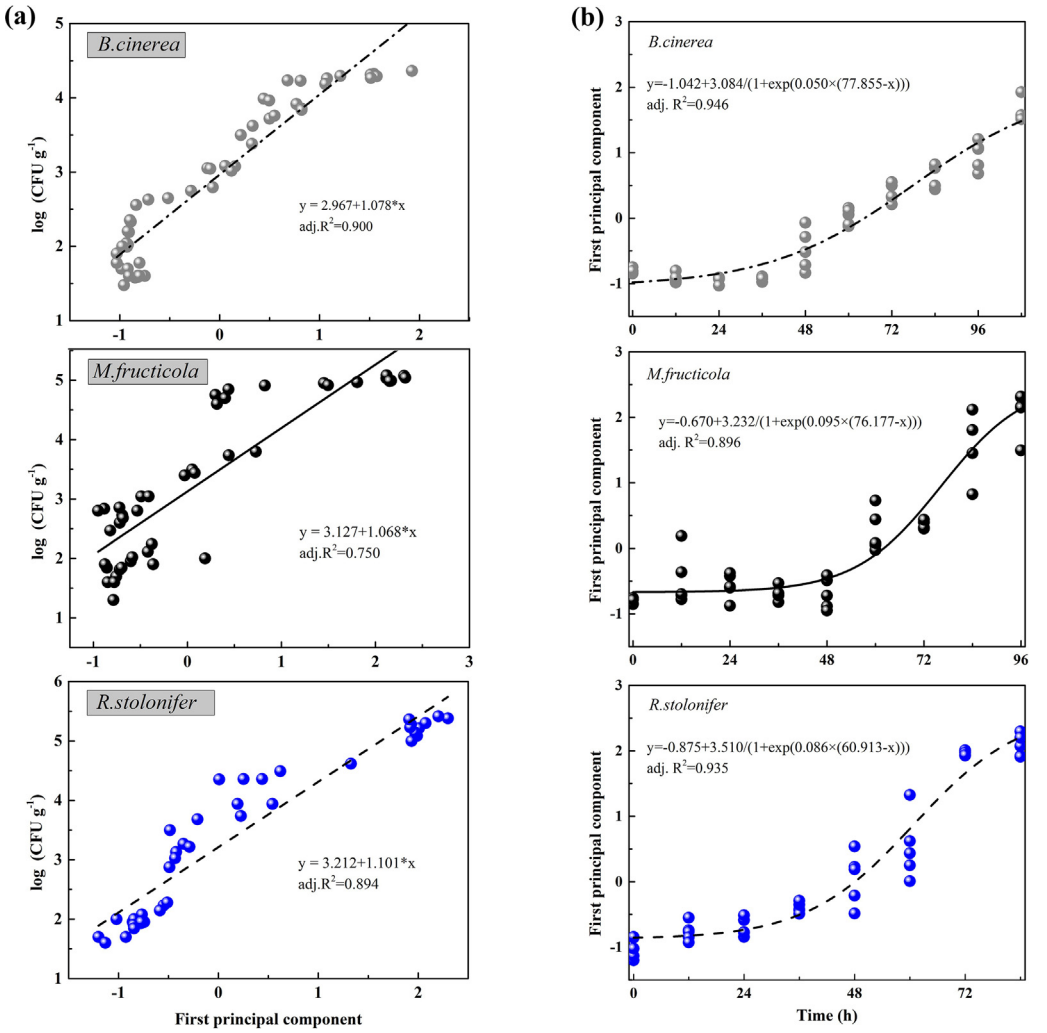

Fig. 3. Correlation between PC1 score and inoculated fungal count (a). Growth simulations of *B. cinerea*, *M. fructicola*, and *R. stolonifer* in peach samples using PC1 data (from E-nose response) (b). ● = *B. cinerea*-inoculated group; ● = *M. fructicola*-inoculated group; ● = *R. stolonifer*-inoculated group, respectively.

accounted for 95.54%, were considered enough information to represent the entire samples (He, Deng & Li, 2006). The data points at different storage periods (0–84 h) were separated and scattered around the graph after PCA processing. The values of PC1 (first principle component) increased with increasing storage period, indicating that the peach aroma, produced as a result of three different fungi infections, is somewhat universal. However, the overlapping at 0, 12, 14, and 36 h suggests that such aroma is slightly different. At 48 h, the samples infected with three fungal species were largely scattered, suggesting that the E-nose sensor responses at this time point (48 h) may be used to discriminate among different fungi-infected peaches.

The loading analysis for each individual fungal species was compared; and the results are shown in Fig. 2. After 24 h storage period, the data points for CK and *R. stolonifer* groups remained largely overlapped, while those for *B. cinerea* and *M. fructicola* groups were slightly overlapped. This suggests that the E-nose responses at this storage period may not be used to discriminate among different fungal species. In contrast, such data points were clearly distinguishable at 48 and 72 h storage periods, indicating that the E-nose responses at after 48 h can be used to discriminate among the four groups of samples.

Because the PCA results were highly linked with the storage periods, the possibility to qualitatively analyze each inoculated fungal groups by PCA was further studied. Correlations between the score of PC1 and inoculated fungal count are shown in Fig. 3a. The results showed that the PC1 score was highly related to the colony counts. The adj. R^2 for *B. cinerea*, *M. fructicola*, and *R. stolonifer* groups were 0.900, 0.750, and 0.894, respectively. The score of PC1 can be utilized to predict the fungal count (after peaches were inoculated with each individual fungus). Three growth simulation models were developed using the score of PC1 from the E-nose response data (Fig. 3b). Comparing all fungal species, the adj. R^2 of *B. cinerea* group (0.946) remained higher than that of *M. fructicola* (0.896) and *R. stolonifer* (0.935) groups. This suggests that the performance of growth simulation for *B. cinerea* is relatively better. As shown in Figs. S2 and 3b, the accuracy (0.050–0.087) of PC1 score simulation was slightly lower than the official analysis method. This might be due to the characteristic of original E-nose data, which was maintained at its maximal limitation in the first principal component (contribution rate = 88.76%), while the residual fungal count data with limited useful information was abandoned. Additionally, it is also likely that this non-destructive method by E-nose is an indirect quantitative assay for fungal count prediction, thus some errors may still exist.

3.6. Colony count prediction feasibility using E-nose signals and PLSR analysis

The feasibility to utilize the E-nose signals obtained from the fungi-inoculated peaches in the prediction of colony count was studied. The independent variable was set as the sensor response, and two-thirds (90 samples) of the total data were selected as a calibration set, while the remaining one-third (45 samples) were used as a prediction set. PLSR analysis was applied to develop models showing correlations between E-

Table 2
Predicted results of colony count on fungal inoculated peach samples by PLSR model.

Species	Latent variables	Calibration		Predication		RPD
		R_c^2	RMSEC (log (CFU g^{-1}))	R_p^2	RMSEP (log (CFU g^{-1}))	
B. cinerea	2	0.907	0.274	0.908	0.235	4.11
M. fructicola	2	0.895	0.422	0.883	0.435	2.80
R. stolonifer	4	0.940	0.216	0.905	0.301	4.16
Total	4	0.831	0.523	0.830	0.527	2.26

Table 3
Discrimination for fungi species inoculated of peaches by PLS-DA model for at 48 h.

Species	Calibration			Predication		
	Samples	Corrected classified	Accuracy (%)	Samples	Corrected classified	Accuracy (%)
CK	30	30	100.00	15	15	100.00
B. cinerea	30	27	90.00	15	13	86.67
M. fructicola	30	25	83.33	15	13	86.67
R. stolonifer	30	30	100.00	15	15	100.00
Total	120	112	93.33	60	56	90.00

nose signals and colony-forming unit (CFU). The statistical parameters for PLSR models are summarized in Table 2. A model that has higher R^2 and lower RMSE (both in calibration and prediction sets) has better performance (Xu, Ye, Wang, Wei, & Cheng, 2017). Comparing the three models for samples in *B. cinerea*, *M. fructicola* and *R. stolonifer* groups in calibration set, *R. stolonifer* group had the highest calibration performance (R_c^2 = 0.940, RMSEC = 0.216 log (CFU g^{-1}), while *M. fructicola* group had the lowest prediction performance (R_c^2 = 0.895, RMSEC = 0.422 log (CFU g^{-1})). Additionally, the accuracy of the model was lower when all data were used as calibration set, whose R_c^2 = 0.831 and RMSEC = 0.523 log (CFU g^{-1}). The prediction results also showed that *B. cinerea* group had the highest R_p^2 (0.908) and lowest RMSEP (0.235 log (CFU g^{-1})) among all three groups. Similarly to that for the calibration set, the models had lower accuracy (R_p^2 = 0.830, RMSEP = 0.527 log (CFU g^{-1})) when all data sets were used as prediction set, compared with that when each individual data was used. The RPD values for all four models were higher than 2.0, indicating that these models can potentially be used in the prediction of colony-forming units in fungi-infected peaches using E-nose response signals.

3.7. Discrimination among individual fungal species using PLS-DA analysis

According to the PCA results (above), which showed that the differences between the four groups of samples could be captured (by the sensors) at 48 h storage period. Hence, new datasets with additional 180 peaches (four groups with 45 samples each) were obtained using E-nose after 48 h post-fungi-inoculation. The PLS-DA algorithm was used to establish the discrimination model (120 samples) for fungal species, and the prediction datasets (60 samples) with individual fungi-infected samples were used as external validations. The results tabulated in Table 3 showed that the discrimination accuracies in calibration datasets for CK, *B. cinerea*, *M. fructicola*, and *R. stolonifer* groups were 100.00, 90.00, 83.33, and 100.00%, respectively. In addition, the prediction accuracies were 100.00, 86.67, 86.67, and 100.00%, respectively for CK, *B. cinerea*, *M. fructicola*, and *R. stolonifer* groups. The CK group was clearly discriminate from the fungi-infected groups, indicating that the model is able to discriminate the infected peaches from the uninfected one. These demonstrations show that E-nose technology can successfully be used to identify individual contaminated fungal species in peaches after 48 h of storage.

4. Conclusion

In the present study, the ability of portable E-nose system, equipped with ten metal oxide semiconductor chemical sensors, in the detection of individual peach-contaminated fungus was evaluated. The statistical results showed that the changes of peaches' volatile compounds were mainly affected by the total amount and the species of fungi. The contents of volatile compounds, including terpenes (e.g., β-myrcene and α-pinene) and aromatic compounds generated during the storage period, were correlated with sensor responses. The sensor responses could be utilized in identifying the three fungal contaminations in peaches. The PCA results also demonstrated that peach samples inoculated with different fungal species during storage could be successfully discriminated after 48 h of storage. The PC1 score was highly related to fungal colony count with adj. R^2 of 0.75–0.90, thus can be used to monitor the growth curve for each fungus in peaches. In addition, colony counts in peach samples were accurately predicted by the PLSR models (R_p^2 = 0.883–0.908, RMSEP = 0.235–0.435 log (CFU g^{-1}), and RPD = 2.80–4.16). Moreover, the discrimination accuracy among the three fungal species was 90.00% at storage time of 48 h. These findings demonstrate that the E-nose technology is a new promising method for the detection of possible contaminations/diseases in peaches.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.foodchem.2018.04.100.

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