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Early detection and classification of pathogenic fungal disease in post-harvest strawberry fruit by electronic nose and gas chromatography–mass spectrometry

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ABSTRACT

Strawberry fruit decay caused by fungal infection usually results in considerable losses during post-harvest storage; thus, discerning the decay and infection type in the early stage is necessary and helpful for reducing the losses. In this study, three common pathogenic fungi belonging to *Botrytis* sp., *Penicillium* sp. and *Rhizopus* sp. were individually inoculated into ripe strawberry fruits; non-inoculated fruits were used as controls. The strawberry fruits were stored at 5 ± 1 °C for 10 days. During storage, inoculated fruits began rotting on day 2, while control fruits began rotting on day 4. The volatile compounds emitted by the fruits were analysed by an electronic nose (E-nose) and gas chromatography–mass spectrometry (GC–MS). Principal component analysis (PCA) showed a clear discrimination in decay on day 0, day 2 and day 4 and the infection type on day 2 after fungal inoculation based on 5 selected sensors of E-nose. The discrimination accuracy of the fungal infection type of strawberry fruits for the four groups reached 96.6% by using multilayer perceptron neural network model. GC–MS results of the four strawberry fruit groups on day 2 identified several key characteristic volatile compounds for each infection treatment, compared with the control. Therefore, E-nose was able to realise the early diagnosis of fungal disease, in addition to an accurate classification of the pathogenic fungal type in the fruits during post-harvest storage.

Introduction

Strawberry is one of the most currently consumed berries and the fifth most preferred fresh fruit in the United States after bananas, apples, oranges and grapes (Boriss, Brunke, & Kreith, 2006). New information on the health benefits of strawberries, owing to its high nutritional values (which include high contents of folate, potassium, vitamin C and fibre), has stimulated domestic consumption rates (Da Silva Pinto, de Carvalho, Lajolo, Genovese, & Shetty, 2010; Seeram, Lee, Scheuller, & Heber, 2006). However, strawberry fruits are highly perishable and vulnerable to tissue damage during harvest and post-harvest handling and storage. The ripe fruits usually have a short post-harvest life, estimated to be less than 5 days due to rapid dehydration, physiological disorders, bruising, mechanical injuries and infections caused by a wide range of phytopathogenic fungi, bacteria and viruses (Mo & Sung, 2007; Sallato, Torres, Zoffoli, & Latorre, 2007). The species usually responsible for heavy losses during storage include those belonging to *Botrytis* sp. (BO), *Penicillium* sp. (PE) and *Rhizopus* sp. (RH) (Nabigol & Morshedi, 2011).

Nowadays, it is difficult to identify fungal diseases owing to which lay in or on a small number of fruits; consequently, the fungal disease spreads to adjacent healthy strawberries in a package unit, resulting in compromised quality and losses during shipping and marketing (Kovach, Petzoldt, & Harman, 2000). Furthermore, conventional microbiological analyses are time consuming, destroy test products and may not provide the immediate results required by the strawberry industry. Therefore, it is crucial for the conventional tests to be replaced by faster, sensitive and directly applicable non-destructive methods, based on the necessity for real-time monitoring of the infection in the early storage stage or during transportation (Papadopoulou, Panagou, Mohareb, & Nychas, 2013).

The electronic nose (E-nose) comprises several electronic gas sensors, which have sensitivity and selectivity to volatile compounds present in the sample headspace of food products. Rather than any specific information on the volatiles detected in the samples, E-nose provides an overall estimate. Many published articles about the application of electronic nose have obtained positive results in food quality control using a pattern recognition algorithm (Peris & Escuder-Gilabert, 2009). Although, the whole chemical volatile profile is analysed and evaluated by E-nose, the composition and content of compound characteristics that are detected by E-nose are usually analysed by gas chromatography–mass spectrometry (GC–MS) (Esposto et al.,

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2006; Lopez De Lerma, Bellincontro, Mencarelli, Moreno, & Peinado, 2012; Olsson, B O Rjesson, Lundstedt, & Schn U Rer, 2002; Pastorelli et al., 2007). The volatile compounds in strawberries have been extensively studied, and more than 360 volatile compounds have been reported (Maarse, 1991). Fruits infected by pathogenic microorganisms produce a different array of volatile compounds, and the compounds characteristic to a specific infection may be assessed by GC–MS (Morales-Valle et al., 2010; Vandendriessche, Keulemans, Geeraerd, Nicolai, & Hertog, 2012). Using E-nose, some preliminary research has been conducted on infections by foodborne microorganisms for fruit juices (Gobbi et al., 2010), beef fillet spoilage (Papadopoulou et al., 2013), onion (Li, Schmidt, & Gitaitis, 2011), blueberry (Li, Krewer, Ji, Scherm, & Kays, 2010) and bread (Needham, Williams, Beales, Voysey, & Magan, 2005). For strawberry fruits, it was found that microbiological activity by yeast could produce metabolites, including acids, alcohols and esters, which change the smell of fruits (Ragaert et al., 2006), but the early detection of infection in stored strawberries by post-harvest fungi using E-nose has not been adequately evaluated.

The present study was designed to investigate the performance of E-nose in the detection and discrimination of strawberry fruits infected by three common post-harvest fungal pathogens in the early storage stage. The differences in volatile compounds observed in infected fruits as well as the controls were further analysed by GC–MS.

Materials and methods

Samples

The experimental samples were intact Hongyan strawberries, hand-harvested in the Nanjing central bar strawberry garden of China in April 2012. The strawberry fruits were selected based on a similar size, maturity and the absence of mechanical injury or infection. Prior to inoculation and analysis, the harvested samples were immediately stored in a controlled chamber at 1 ± 1 °C and 90% relative humidity. For surface sterilization, the strawberry fruits were immersed in 75% ethanol for 30 s (Li et al., 2010). Subsequently, prior to inoculation, the strawberry fruit surfaces were washed three times in sterile distilled water to remove the ethanol residue.

Three pathogenic fungi, namely *Botrytis* sp. (BO), *Penicillium* sp. (PE) and *Rhizopus* sp. (RH) were used for inoculation. The fungi were grown on potato dextrose agar (PDA) at 24 °C and 85% relative humidity for 7 days prior to inoculation experiments.

Inoculation

Three treatments of fungal inoculation were performed in a bacteria-free operating environment. Strawberry fruits were immersed in spore suspensions (4×10^5 spores/mL) of the three fungal pathogens (or in sterile distilled water as a control, CK) for 30 s. Following evaporation of surface moisture from the fruits, 78 samples for each group were placed in a 150-mL beaker wrapped with a single layer of polyethylene plastic film and stored at 5 ± 1 °C for 10 days. Each sample had three strawberry fruits weighing approximately 50 ± 5 g. Sixty samples for each treatment and the control were used for E-nose measurements on day 0, day 2, day 4, day 6, day 8 and day 10. Three samples were handled and used for GC–MS, with three replicates at each time interval (nine strawberry fruits, and total of 54 strawberry fruits for 6 tests). E-nose measurements and GC–MS for day 0 were conducted 1 h after inoculation and the other tests were processed every 2 days for a total of six times.

E-nose

Volatiles from the three inoculation treatments and the control sample were acquired by a portable E-nose (PEN 3, Win Muster Air-sense Analytics Inc., Germany). An E-nose system consists of a sampling unit, a detector with an array of 10 different metal oxide sensors and pattern recognition software (Win Muster v.1.6) for data recording and analysis. Each sensor is usually sensitive to several different volatile compounds. The general description of every sensor was as follow: S_1, sensitive to aromatic compounds; S_2, sensitive to nitrogen oxides; S_3, sensitive to ammonia and aromatic compounds; S_4, sensitive to hydrogen; S_5, sensitive to alkenes and aromatic compounds; S_6, sensitive to methane broad range; S_7, sensitive to sulphur compounds; S_8, sensitive to alcohols and partially aromatic compounds; S_9, sensitive to aromatics compounds and sulphur organic compounds; and S_{10}, sensitive to alkane (Gomez, Wang, Hu, & Pereira, 2008; Liu et al., 2012). Clean air filtered by activated charcoal was used to wash all the sensors for 80 s prior to every test and was helpful to keep the sensors sensitive to the volatile compounds. The values were selected at the flat area of the responsive curves in every measurement. A strawberry sample was acquired from the refrigerator and placed in the box at 24 ± 1 °C and 85% RH without sealing. After 2 h, when the temperature of the fruit reached room temperature (24 °C), the mouth of the beaker with the strawberry fruit was sealed with tinfoil and the volatile compounds in the headspace were equilibrated for 10 min at room temperature (24 °C). A 3-mm Teflon tubing connected to a needle pumped headspace gas in the sensor chamber at a constant rate of 120 mL/min. Impaling the tinfoil during the measurement absorbed the gas accumulated in the beaker. Following gas entry in the sensor chamber, the conductance ratio of the 10 sensors changed. The sensor response was acquired by the G/G_0 ratio, where G_0 and G stand for the conductance of the metal oxide sensor combined with clean air and the sample gas, respectively. The software coupled with E-nose regulated the entire procedure. The detection time was set as 60 s, which was sufficient to obtain a stable response curve for the sensors. Following each measurement, there was a cleaning phase for 80 s, and an automatic zero setting phase for 5 s prior to the next detection. All data was recorded and stored by the computer. In this experiment, the response curve stabilised after 25 s and the value at 30 s was subsequently used.

Decay index

Strawberry fruit decay during storage can reflect fungal infection (Zhao, Wang, Tu, & Liu, 2011). The fungi decay of strawberry fruit for each of the four groups was inspected visually by a panel of 10 trained persons according to a 3-grade scale, where 0 = no decay; 1 = slight decay (covering <25% of the fruit surface) and 2 = moderate decay (covering ≥25% of the fruit surface). The panellists gave the score of every sample after discussion among them. The decay index was computed by the following formula: $[(0 \times N0 + 1 \times N1 + 2 \times N2) \times 100 / (2 \times N)]$, where N is the amount of measured fruit and N0, N1 and N2 are the respective numbers of fruits for each grade scale corresponding to the severity of the disease (Liu et al., 2010).

Headspace solid phase micro-extraction and GC–MS

The volatile compounds of strawberry fruits were collected and analysed by headspace solid phase micro-extraction and gas chromatography–mass spectrometry (HS-SPME–GC–MS). Following E-nose analysis, the strawberry fruits in each of the three replicates were cut up and blended for volatile gas identification and quantification. The volatiles in the sample headspace were extracted and concentrated using a SPME fibre (poly-dimethylsiloxane, PDMS, 100 μm, Supelco, USA), separated and identified by GC–MS (7890A/5975C, Agilent, USA).

SPME fibre was aged in the GC inlet port at 250 °C for 30 min at 1 mL/min in order to remove the residual gas. Approximately 10 g of strawberry fruits from one sample was weighed and placed in a 20-mL vial. The volatile was equilibrated at 40 °C for 40 min in the vial sealed with a PTFE/BYTL septum and absorbed by the extraction head of SPME from the vial. Following equilibration, the extraction head was injected into the GC inlet port in a split-less mode. Subsequently,