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Detecting sour skin infected onions using a customized gas sensor array

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ABSTRACT

The overall goal of this study was to test a customized gas sensor array in its ability to detect an important postharvest disease (sour skin) in onions. The sensor array consists of seven metal oxide semiconductor gas sensors and a microcontroller-based automatic data logging system. Three features were extracted from the sensor responses and three baseline correction methods were employed to correct the sensors' responses. The gas sensor array was tested in two separate experiments with two treatments (control and sour skin). The multivariate data analysis revealed that the "relative response" feature combined with relative baseline correction method provided the best discrimination of infected onions among healthy ones. The best performance (85%) was achieved by using the support vector machine model when the data collected from an independent experiment were used for validation. The study demonstrated the potential of a gas sensor array to detect sour skin-infected onions placed among healthy onions in storage.

1. Introduction

Onion is the second most economically important commercial vegetable crop for fresh market in the United States (USDA-NASS, 2013). It is estimated that approximately 6.2 billion pounds of onions are produced each year in the U.S. Across the world, average annual onion consumption per person is estimated to be over 13 lb (Natinal Onion Association, 2011). Onions like any other vegetables are threatened by various bacterial or fungal diseases. This study focuses on detection of a post-harvest bacterial disease, called sour skin, caused by the bacteria *Burkholderia cepacia*. The pathogen survives in the soil and is splashed onto the leaves and into the neck of the onion during rain or overhead irrigation. The presence of wounds and water soaked tissue provides an opening to these bacterial cells into the onion. Warm weather (~30 °C) and high humidity promotes bacterial growth resulting in brown and water soaked scales under the first or second onion layer (Schwartz and Mohan, 2007). During the earlier stages of bacterial infection, symptoms are limited to a very small portion of inner scales of the onion. Hence, it is difficult to sort the infected and healthy onions by human visual inspection before storage (Gitaitis and Tollner, 2005). The lack of effective detection of diseased onions often leads to spreading of infection among onions during cold storage resulting in up to 50% losses in some individual storage houses (Gitaitis and Tollner, 2005; Tollner et al., 1995).

One study using gas chromatography–mass spectrometry (GC–MS) found that onions infected by two pathogens (*B. cepacia* and *Burkholderia alli*) emitted unique VOCs both qualitatively and quantitatively (Li et al., 2011), compared to healthy onions. These differences in volatile compounds could be used as the indicator of pathogen infection. Unlike GC–MS that identifies individual volatile compounds, a gas sensor array (also known as electronic nose or E-nose) provides information on the overall differences in volatile profiles of different samples. The E-nose consists of an array of gas sensors and a gas delivery system which delivers the volatiles to the gas sensors. The gas sensors react to the volatiles based on their concentration and output a voltage signal accordingly. The voltage signals (or so called "smellprint") can be processed and analyzed using pattern recognition algorithms of the E-nose system to categorize the sample.

Since the early 1980s when the concept of E-nose was first proposed (Persaud and Dodd, 1982), various commercially available and custom-made E-noses have been developed and applied to diverse areas such as quality control for food and beverages (Baldwin et al., 2011; Casalinuovo et al., 2006), environmental monitoring (Romain and Nicolas, 2010; Wilson, 2012), and medical diagnosis (Di Natale et al., 2003; Schiffman et al., 1997). Most applications of the E-nose so far have been in the food industry and the E-nose technology has shown its potential to detect the spoilage induced by fungal or bacterial diseases in various foods

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(Röck et al., 2008; Wilson, 2013). For instance, The electronic nose has been shown to be effective in detecting fish spoilage because spoiled fish emit unique volatile compounds due to metabolic activities of microorganisms (Di Natale et al., 2001b; Olafsdottir et al., 2004). A custom-made gas sensor array (called FishNose) was used to monitor quality changes of smoked salmon during storage. The E-nose system achieved classification accuracy of 93% for spoiled fish. The system was also able to predict other quality properties such as sweet/sour and off odour, as well as microbial counts (Haugen et al., 2006; Olafsdottir et al., 2005). Meat could be stored for a long period of time before reaching to consumers and it could develop bacterial spoilage during storage. Several studies have been done using the electronic noses to detect beef spoilage and relate the gas sensor readings with the bacteriological analysis (Balasubramanian et al., 2009; El Barbri et al., 2008; Ghasemi-Varnamkhasti et al., 2009). The early detection of quality changes in cereal grains is critical to the grain industry. Commercial electronic noses were able to detect fungal infected grain samples which had 6–8 times higher concentration of trichodiene, a precursor for the formation of fusarium metabolites (Perkowski et al., 2008). Electronic noses have been used for fruit and vegetable quality assessment, such as detecting and classifying post-harvest diseases in blueberry fruit using the Cyranose 320 (Li et al., 2010), mango fruit rot detection using an ultra fast GC (zNoseTM) (Li et al., 2009b), detecting the presence of soft rot in stored potato tubers using multiple gas sensors (de Lacy Costello, 2000), as well as apple and orange mealiness and skin damage by a quartz resonators based electronic nose (Di Natale et al., 2001a). A recent study demonstrated the success of a custom-made electronic nose for biodeterioration of oranges infected by fungal disease *Penicillium digitatum* (Gruber et al., 2013). In particular for onions, a conducting polymer based electronic nose was used for evaluation of onion bulb quality (Abbey et al., 2004) and discrimination amongst members of the genus Alliums (Abbey et al., 2001). As the past studies have documented that fungal or bacterial infected onions emitted different volatile compounds than healthy onions (Li et al., 2011; Prithiviraj et al., 2004), our group conducted studies using a commercial electronic nose (Cyranose 320) for onion postharvest disease detection and proved the feasibility of the method (Li et al., 2009a, 2011).

Commercial gas sensing instruments, however, are manufactured for general purposes without targeting a specific application. In addition, they are usually expensive (in several thousand dollars) and therefore not feasible to be deployed in a large onion storage room with multiple units. In addition, data collection and analysis in commercial electronic noses lack of flexibility and is hard to be automated in onion storage. To address these issues, our group recently developed a customized electronic nose system using seven metal oxide semiconductor (MOS) gas sensors for onion postharvest disease detection in storage (Konduru et al., 2015). The MOS sensors were chosen due to their advantages compared to other types of gas sensors: they are easy to operate, readily available at a low price and are designed to be sensitive to a wide range of compounds with high sensitivity. The customized gas sensor array was developed with about $300 in parts, a fraction of what a commercial electronic nose costs. Additionally, data preprocessing and feature extraction could be optimized for the specific application of onion postharvest disease detection.

The main goal of this study was to test the automated customized electronic nose system in detecting the presence of sour skin disease in onions. The specific objectives of this study were to (1) Compare three baseline correction methods and three features for data pre-processing. (2) Conduct principal component analysis (PCA) and develop classification models to distinguish healthy and sour skin infected onions. (3) Select the best combination of MOS sensors from the seven available sensors.

2. Materials and methods

2.1. Preparing onion samples

Two groups of Jumbo yellow onion bulbs were purchased from a local grocery store and the two groups were from the state of Oregon and Washington in the United States, respectively. Only onions without major bruises and damages were selected. To avoid any unwanted plant pathogen harbored on the dry skin, the dry skin of the onions was peeled off and the surface of bulbs was sterilized with 70% ethanol solution and allowed to stand for 10 min before washing with distilled water to remove chemical residues. The moisture on the onions was wiped clean using a clean paper towel. The onions were then dried by placing them on a clean surface for 1 h at room temperature (24 ± 2 °C).

2.2. Inoculation and incubation

Cultures of *B. cepacia*, strain Bc 98-4, were obtained from the Natural Products Laboratory at the University of Georgia, Tifton. The cultures were grown on tryptic soy agar after incubating for ~48 h at 30 °C. The petri plates were then placed in room temperature (24 ± 2 °C) to slow down the growth of bacteria. Using a sterile loop, the bacterial colonies were transferred carefully to a vial containing 45 ml of distilled water. The vial was thoroughly shaken until the bacterial colonies were mixed with water and was indicated when the bacterial solution turned turbid.

Using an Eppendorf Biophotometer 22331 (Eppendorf, Hauppauge, NY, USA) spectrophotometer, an approximate number of bacterial cells was measured based on the quantitative measurement of the transmission and reflection properties. It was estimated that there were $\sim 5 \times 10^8$ bacterial cells per 45 ml of solution. Using a 3 ml sterile syringe (Becton Dickinson and Company, Luer-Lok Tip, Mexico), 1 ml each of bacterial inoculum was injected on two opposite sides of the neck region of the onion by inserting the needle ~30 mm deep at an approximate angle between 45° and 55° from the base. Pressure was applied on the inoculated region using the thumb finger to make sure the bacterial solution did not flow back through the injected region.

A tray of labeled infected onions were placed in an incubator for 48 h at 30 °C which was the optimum growth temperature to facilitate sour skin infection in the onion samples. Similarly, the control onions were prepared by injecting 1 ml of distilled water into each of the bulbs. The sour skin infected onion samples and control onions were placed in a clean room maintained at room temperature (24 ± 2 °C) for 3 h prior to placing the onions in a clean box and sealed using a thin strip of parafilm. After conditioning, the onions were allowed to stand for 6 h for the volatiles to accumulate in the headspace before they were analyzed by the gas sensor array.

2.3. Experimental design

The experiment was conducted in two batches. In each batch, sixteen clean plastic boxes were used, in which 8 boxes (also referred to as replicates) were allocated for control onions and the remaining 8 boxes (also referred to as replicates) were used for sour skin infected onions. The volume of the sample container was 6.7 l with a dimension of 10 × 13 × 4 in. Each control had three healthy onion bulbs (intact onions without being injected with water) and two control onion bulbs (onions injected with water); each infected treatment had three healthy onion bulbs and two sour skin infected onion bulbs. The volatiles released by the onions in each box were measured three times from 3 day after inoculation (DAI) through 7 DAI for both batches, except 3 and 4