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Detection of onion postharvest diseases by analyses of headspace volatiles using a gas sensor array and GC-MS

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ABSTRACT

Onion postharvest diseases cause significant losses in storage. Volatile sensing by the gas sensor array technology could be used as a promising alternative method to detect onion diseases. Onions were inoculated with *Botrytis allii* and *Burkholderia cepacia*, causal pathogen for Botrytis neck rot and sour skin, respectively. In the first phase of this study, 30 onions with equal number of *B. allii* inoculated and control healthy onions were measured by the gas sensor array from 8 to 11 days after inoculation (dai) and the principal component analysis (PCA) score plot demonstrated that the gas sensor array responded differently to Botrytis neck rot infected onions from those of healthy onions. In the second phase, 30 onions with 10 for each of the three treatments (Botrytis neck rot, sour skin, control) were measured by the gas sensor array on 5, 6, and 7 dai. The PCA score plot illustrated that three treatments formed three distinct clusters, while a hierarchical cluster analysis dendrogram indicated that the response of the gas sensor array to Botrytis neck rot and sour skin were similar. The correct classification rate of the linear discriminant model for three treatments was over 97.8%. Results from GC-MS showed that total 24 major volatiles were identified from the headspace of three treatments. Sixteen compounds were uniquely present in *B. allii* and *B. cepacia* inoculated onion bulbs. Total amount of volatile compounds detected in pathogen inoculated bulbs was one to two orders of magnitude higher than that of control healthy bulbs. This study demonstrated the feasibility of using a gas sensor array to detect two onion postharvest diseases in storage.

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1. Introduction

Onions are the third most valuable fresh vegetable crop in the U.S. (following only lettuce and tomato) and the second most valuable vegetable in the world (following only tomato) (National Onion Association, 2008; USDA-NASS, 2006). Almost 60% of non-processed onions in the nation are put in storage and consumed weeks or months later to extend the season and capitalize on a more favorable market window (Burden, 2008; National Onion Association, 2008). Normally, onions can be stored for several months with a marketable quality in a cold, dry, and well ventilated room. However, fungal and bacterial diseases affect stored onions and cause substantial losses in storage. Outbreaks of these fungal and bacterial diseases are usually caused by a few damaged and infected onions which eventually spread the pathogen and spoil to nearby wholesome onions in storage. Botrytis neck rot (caused by fungus *Botrytis allii*) and sour skin (caused by bacteria *Burkholderia cepacia*) are two major onion diseases (Schwartz & Mohan, 2008, p. 127). Botrytis neck rot infects onion bulbs from the neck to inner layers; the sour skin usually displays the symptom with brown and water soaked main scales under the first one or two layers. Due to the nature of these two pathogens, they are virtually undetectable by human visual inspection which is a common practice in most onion packing houses. Because no effective detection methods are available, onion handlers are unaware of the presence of these diseases in the early stage until onions exhibit visual symptoms that make them unsalable at the end of the storage period. For instance, Botrytis neck rot could cause as high as 50–70% storage losses in some years (Boyhan & Torrance, 2002; Ceponis, Cappellini, & Lightner, 1986).

In order to reduce postharvest losses, an effective detection technology that can identify pathogen infected onions in storage would be of great value to the onion industry. It is well known that compositions in volatile organic compounds (VOCs) may vary when fruit and vegetables experience diseases, physical damages, or physiological changes such as ripening (Simon, Hetzroni, Bordelon,

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Miles, & Charles, 1996). One study has shown that the volatile profile from *B. allii* inoculated onions is different from sterile water-inoculated onion bulbs (Prithiviraj, Vikram, Kushalappa & Yaylayan, 2004). Another study proved that a gas sensor array could detect and differentiate three types of blueberry postharvest diseases by analyzing their headspace gas (Li, Krewer, Ji, Scherm, & Kays, 2010). Unlike gas chromatography-mass spectrometry (GC-MS) or a human sensory panel, a gas sensor array offers an alternative method for rapid and inexpensive detection of volatile patterns. The gas sensor array, also known as the "electronic nose" (E-nose), is a chemical sensing and identification device that provides a rapid method of differentiating volatile profiles instead of identifying individual volatile compounds as GC-MS does (Mandelis & Christofides, 1993; Schaller, Bosset, & Escher, 1998). Since the invention of the E-nose technology in the early 1980s (Persaud & Dodd, 1982), this concept has been studied extensively and many industrial applications of the gas sensor array already have been put into practice. For instance, gas sensor array-based technology has been used for lung cancer screening in clinical trials (Machado et al., 2005), fire and ammonia detection in spacecraft (Young, Buttner, Linnell, & Ramesham, 2003), as well as quality control in the food industry (Mielle & Marquis, 2001). In particular, the E-nose was applied to an at-line monitoring and controlling the aroma during the drying process of Iberian hams in chambers (Abass & Coper, 1999), as well as at-line quality sorting of spoiled sugar beet tubers (Kaipanen, 1998). This technology was also used for Allium research. The AromaScan E-nose was used to differentiate Allium species and growing conditions in the past (Abbey, Aked, & Joyce, 2001; Abbey, Joyce, Aked, & Smith, 2005). A recent study has proven that a conducting polymer sensor based E-nose could respond differently to sour skin infected onions from the wholesome onions (Li, Gitaitis, Tollner, Sumner, & MacLean, 2009). Several studies have been done to identify headspace volatile compounds emitted by pathogen inoculated potato tubers and carrots (de Lacy Costello et al., 1999; Vikram, Lui, Hossain & Kushalappa, 2006). To our knowledge, however, there are no reports in the literature examining volatiles in sour skin inoculated onion bulbs and little work has been done to investigate the E-nose's capability to detect Botrytis neck rot and sour skin in onions. The overall goal of this study was to explore whether the E-nose can detect and differentiate Botrytis neck rot, sour skin, and healthy onion bulbs by measuring their headspace volatiles, as well as to characterize volatile profiles of three onion treatments by using the GC-MS.

2. Materials and methods

2.1. Plant materials and inoculation

Vidalia sweet onion bulbs cv. Nirvana harvested in April 2008 were used for this study. The onion samples were picked at optimum maturity when ~80% of the necks were soft enough for leaves to collapse. Onion samples were stored in a cold room at 4 °C (R.H. 80%) for about 4 weeks before they were tested. Before use, dry skins were removed, basal roots were trimmed and the bulbs were surface sterilized using 1.29×10^4 mol/m^3 ethanol. Onions were then washed with sterilized distilled water to remove chemical residues. Cultures of *B. cepacia*, strain Bc 98-4, were produced on tryptic soy agar after incubating at 30 °C for ~48 h. Cultures of *B. allii*, strain Ba 09-1, were produced on potato dextrose agar (PDA) after incubating at ~ 22 °C for ~ 148 h. Bc 98-4 was stored and maintained in 2.06×10^3 mol/m^3 glycerol at $\gtrsim -80$ °C. Ba 09-1 was stored and maintained on both diseased onion bulbs and PDA plates at ~4 °C. A square (1 cm × 1 cm) of onion flesh was cut out with a depth of 6.5 mm using a razor blade and a similar size agar containing *B. allii* culture was removed from the inoculum plate and filled the hole in the onion. Every effort was made to use agar plugs of the same size which would provide a similar concentration of Botrytis for each bulb. In total, four holes were cut along the equatorial line of the bulb with equal distance. Similar physical wounds were made in control onions using a razor blade but without filling the *B. allii* inoculum. Sour skin inoculation was created by stabbing *B. cepacia* contaminated sterile wooden toothpicks into bulbs. The detailed method for sour skin inoculation was presented by Li et al. (2009).

The control and Botrytis inoculated onions were stored in the air conditioned room at 24 ± 2 °C, while sour skin inoculated onions were placed in an incubator at 30 °C (the optimal growth temperature for the sour skin). *B. allii* inoculated onions were placed in a plastic container sealed by the aluminum foil, where a high humidity environment was created to facilitate the growth of the fungus by wet paper towels in the container. Onion bulbs with different treatments were placed in glass jars with 2 L volume and 89 mm wide mouth covered by a square of aluminum foil (100 × 100 mm). The contained onion samples were placed under room temperature (24 ± 2 °C) for 12 h before each measurement to allow the headspace gas to reach the equilibrium. Therefore, the temperature should not be a factor when the volatile compounds were analyzed for the three treatments.

2.2. Experiment design

Experiments were divided into two phases. The first phase was to investigate whether the E-nose could differentiate Botrytis neck rot and healthy onions; the second phase was to explore whether the E-nose could delineate volatile profiles from three treatments: Botrytis neck rot, sour skin, and healthy onions. In the first phase, two experiments were conducted in order to prove the repeatability of the E-nose. In the two experiments, 15 onions were used for control healthy onions and 15 onions were inoculated by the *B. allii*. Onion samples were measured on 10 and 11 dai in the first experiment, and 8 and 9 dai in the second experiment. Total 177 data sets were collected in each experiment. In the second phase, 10 onions were used for each of three treatments (control, Botrytis neck rot, and sour skin) which were measured on 5, 6, 7 dai and total 270 data sets were combined for statistical analysis. Each onion headspace was measured twice and the average of these two measurements was used as the third measurement.

2.3. Gas sensor array and data acquisition

Headspace samples from three onion treatments (control, sour skin, and Botrytis neck rot) were analyzed with a hand held E-nose which contains 32 conducting polymer sensors (Smith Detection Inc., Pasadena, CA). The characteristics of the gas sensor array were described in a separate paper (Li et al., 2009). During the sampling process, the E-nose sampling needle was inserted into the concentration chamber to draw headspace volatiles. Each sampling process took about 2 min. The conducting polymer sensors within the E-nose were purged by the ambient air between each measurement. The raw sensor resistance values were normalized to improve the signal to noise ratio by embedded data preprocessing algorithms in the E-nose. The preprocessed data were saved in a Microsoft Access file (Microsoft Inc., Redmond, WA) for further statistical analyses and pattern recognition algorithm development.

2.4. Data analysis

Principal component analysis (PCA), a multivariate data analysis technique, was used to reduce the dimensionality of the E-nose data from 32 to a few major principal components (Krzanowski,