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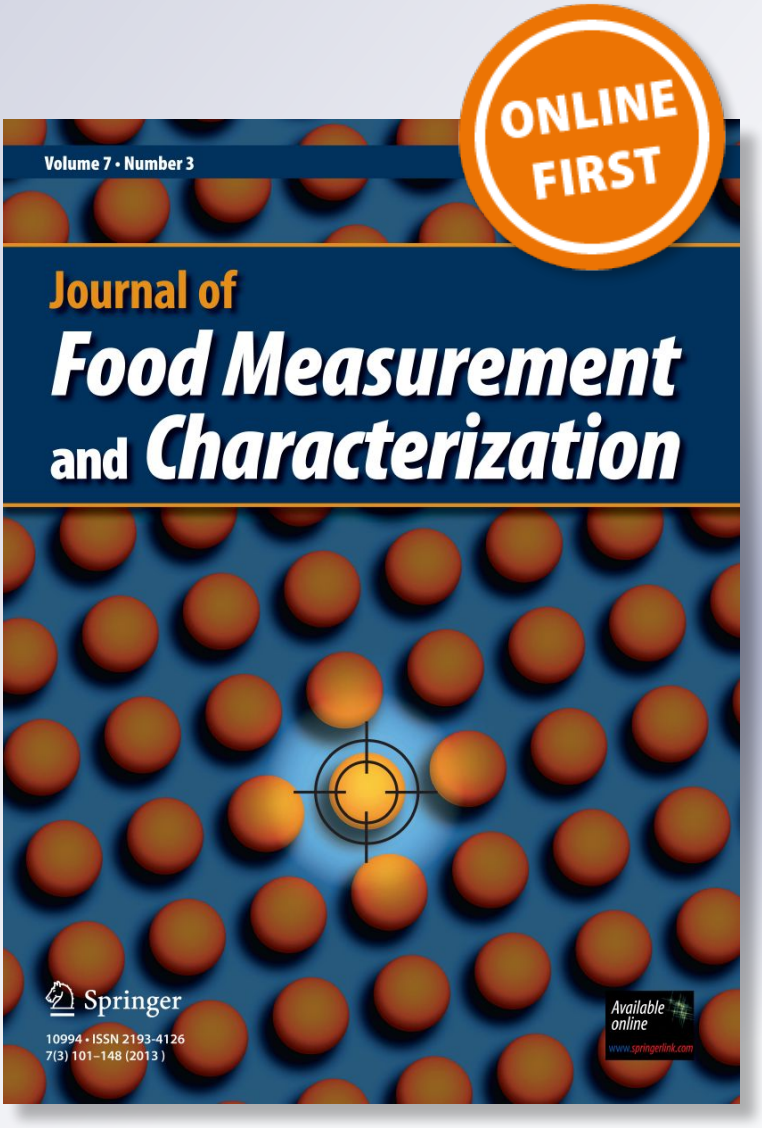

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ORIGINAL PAPER

FAIMS based sensing of *Burkholderia cepacia* caused sour skin in onions under bulk storage condition

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Abstract Onion (*Allium cepa* L.) is one of the most important vegetable crops in the Pacific Northwestern states of the U.S., and is stored for 9–12 months under bulk storage condition. Portable non-contact sensing technologies are needed to effectively detect pathogenic infections to the onion bulbs during bulk storage periods for real-time crop loss management. Therefore, portable field asymmetric ion mobility spectrometry (FAIMS) was evaluated towards detection of onion sour skin caused by *Burkholderia cepacia*, an important postharvest disease under bulk storage conditions. Studied were onions stored at 25 °C under two treatment conditions, inoculated with *B. cepacia* and with sterile water (as control), during 21-days temporal storage. Results confirmed applicability of FAIMS to detect volatile organic compounds pertinent to *B. cepacia* caused sour skin as early as 3 days after inoculation. Principal component analysis (PCA) score plots for FAIMS extracted data illustrated distinct clusters corresponding to inoculated and healthy treatments. PCA also suggested a significant range of dispersion field intensity (47–77%) and compensation voltage (−0.24 to 0.48 V), which could potentially be used to train portable FAIMS for real-time detection of sour skin under bulk storage conditions.

Keywords Onion storage · Sour skin · VOCs release · Early disease detection

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Introduction

Onion (*Allium cepa* L.) is one of the most important vegetable crops in the world and the U.S. is the third largest producer of dry onion. The total annual production of onions in the U.S. averaged to 3.4 million MT for the period of 2004–2014, contributing around 12.3% of the total global production [1]. A large part of the produce gets stored under controlled condition in bulk storage units for up to 9 months after curing to extend the period of crop availability. The optimum temperature range for storage is 1.1–7 °C at a relative humidity of 65–70% [2, 3]. Under such conditions, the crop is highly susceptible to various infectious diseases like *Botrytis* neck rot (soft rot) caused by *Botrytis allii* and sour skin caused by *Burkholderia cepacia* [4]. Such infections can potentially cause a crop loss as high as 50–70% under storage conditions [5]. *B. cepacia* caused sour skin in onion bulbs is one of the most common bacterial infections under storage conditions in Pacific Northwest. Bruises and wounds on the bulb surface, high humidity, elevated temperature and poor ventilation accelerates growth of bacterial infection that can rapidly spread through the onions under bulk storage.

Detection of sour skin caused by *B. cepacia* is of prime importance for growers as well as storage managers. Existing detection methods are based on visual and olfactory symptoms, often leading to very late detection. By the time symptoms are visible or sensed through subjective human olfactory system, the infection may have caused significant crop loss. Plants and produce release volatile organic compounds (VOCs) both in normal as well as stressed conditions [6–8]. However, plants either release entirely different VOCs or different concentrations of VOCs when stressed. These stresses can occur because of limited plant water availability, temperature change, attack of predators and

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plant diseases etc. [9, 10]. Produce stored under bulk storage conditions also produce VOCs characteristics of such stresses [11–13]. Gas chromatography–mass spectrometry (GC–MS) and GC-flame ionization detection (FID) techniques have been used to detect and characterize the pertinent VOCs. Such methods have been employed for detecting storage infections in potato [14], carrot [15] and onion [5]. Electronic noses (e-nose), mimicking mammalian olfactory symptoms, have also been developed and used to detect the infections of produce under bulk storage conditions [16–18]. Above methods have limitations towards VOCs sensing under bulk storage conditions. GC–MS/GC-FID based sensing is highly accurate, sensitive and precise, however the method is time-consuming, expensive, requires skilled labor and lacks field portability required in real-time disease detection. E-noses, comprising of conducting polymers [5] and metal oxide sensors [18] are sensitive to humidity and temperature, and can be overloaded by certain analytes, causing limited sensor life and poor precision [19]. Having a gas analysis system which could identify sour skin at early stages could aid greatly to the onion industry in the U.S. and worldwide.

An emerging technology for gas analysis, field asymmetric ion mobility spectrometry (FAIMS) (Fig. 1) finds potential applications in food, dairy [20], health [21] and agriculture sectors [22–24]. Recent studies have explored the application of FAIMS towards early detection *Pectobacterium carotovorum* subsp. *carotovorum* caused soft rot in stored potatoes [23, 24]. Expanding the FAIMS application domain, this study evaluated FAIMS towards potential detection of key volatile biomarkers associated with *B. cepacia* that cause sour skin in onions.

The overall goal of this research was to study the pattern of VOCs released by onion bulbs inoculated by *B. cepacia* under bulk storage conditions. It was hypothesized that portable FAIMS system can detect sour skin associated VOCs before persistence and subsequent detection of the visual and olfactory symptoms. Such approach can potentially lead towards rapid detection of the infection and will result in effective crop loss management. Specific objectives of the study were to evaluate the applicability of portable FAIMS towards: (1) detection of VOCs pertinent to *B. cepacia* in stored onions, and (2) assessing the temporal stage of disease detection, through quantification of released VOCs.

Materials and methods

Sample preparation

Onion bulbs of Vaquero cultivars (yellow medium storage onions) are one of the most important storage cultivars and top trading variety of onions grown in the Pacific Northwest [25]. Onion bulbs of Vaquero cultivars used in this study were procured from a local grocery store and were harvest of 2015 season. Extra care was taken to select onions with no bruises or damages, and the dry skin was removed from the onions. Study involved two treatments with three replicate samples, each having three onion bulbs in a glass jar. Treatment-1 and -2 had bulbs inoculated with sterile water and *B. cepacia* respectively, and kept at room temperature (around 25 °C) throughout the study.

Burkholderia cepacia strain BcWSU1 was grown overnight in 5-ml of nutrient broth at 28 °C with agitation. Details of the inoculum preparation can be found in Schroeder and Humann [26]. The prepared inoculum was dispensed into 15 ml sterile test tubes. A 0.5 ml aliquot of the inoculum was injected into each onion bulb, using a 22-gauge needle, following a methodology reported in Schroeder and du Toit [27]. For each of the replicate of inoculated treatment, a new inoculum sterile tube and sterile syringe was used to avoid cross contamination. Before inoculation, the injection site was sterilized by cleaning the surface using a cotton swab with 95% ethanol. A similar

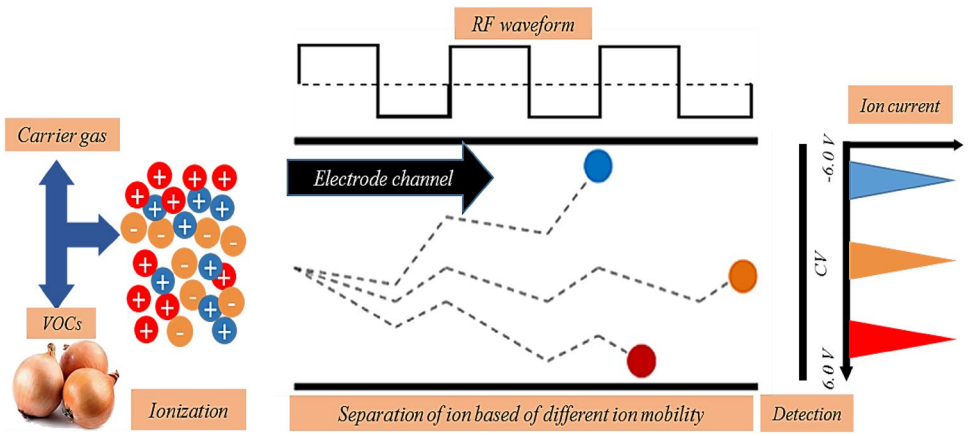

Fig. 1 Depiction of FAIMS working principle towards detection of pertinent VOC biomarkers associated to onion sour skin

protocol was followed for inoculating a set of three onion bulbs with sterile water, which served as healthy controls.

Sampling module and data acquisition

To sample the VOCs from *B. cepacia* inoculated and healthy control, a custom sampling module was fabricated. Glass jars [capacity 1.0 gallon (3.79 l), Specialty Bottles, WA, U.S.] were used to store the samples during 21-days storage period. Each sample jar was sealed with food grade cling wrap film during the storage to create aerobic conditions mimicking bulk storage environment.

In this study, portable FAIMS (Owlstone Nanotech Ltd., Cambridge, UK) was used to evaluate the samples. Details of the customized module and working of the integrated unit are in Sinha et al. [24]. Pertinent to onions, sampling was done every day for a week (0–6 day after inoculation, DAI) and then every 5 days till 21 DAI. First phase of sampling, with 1-day sampling resolution, was done to assess the time frame of sour skin detection, while the second phase monitored the temporal progression and pertinent changes in the VOCs release. The VOCs pertinent to healthy and *B. cepacia* inoculated onion bulbs were captured through FAIMS in form of DF scans, which are the 3D matrix of ion current values corresponding to changing dispersion field (DF) intensity and compensation voltage (CV). A total of six DF scans were collected for each sample on each of the sampling days (i.e., 6 DFs/sample × 3 samples/treatment × 2 treatments).

Data analysis

The FAIMS unit conducts the volatile (gas) analysis in both positive and negative modes. Out of the six scans obtained, only two middle scans in positive mode, where ion current values were found stable, were used for further analysis. Stability of the ion current was evaluated using Lonestar® software (Owlstone Nanotech Ltd., Cambridge, UK). For data analysis, only the positive mode DF matrices were used as this model predominantly exhibited the differences among the treatments. In each of the DF matrix, the intensity of DF changes from 0 to 100% in 51 steps and CV changes from −6 to 6 V in 512 steps, creating a 51 × 512 matrix of ion current values. Visual inspection of the ion current plots (DF matrices) confirmed a DF range of 40–100%, pertaining to maximum differences between healthy and inoculated treatments. For each of the DF intensity in the abovementioned range (31 values), the maximum ion currents were extracted as features.

The FAIMS response towards *B. cepacia* caused sour skin in onions was analyzed using Principal component analysis (PCA) in two different phases. PCA is a non-parametric technique for multivariate data analysis, which extracts the relevant features from a large set of interdependent variables by reducing the dimension of FAIMS data from 31 values to a few principal components (PCs) [28]. For the first phase, the day wise FAIMS response was cumulated for PCA. The resulting dataset consisted of 120 samples (2 treatments × 3 replicates/treatment × 10 FAIMS scan days × 2 scans per day) with 31 different DF values, i.e., 120 × 31 size matrix. For the second phase, FAIMS response for each day was considered. For each DAI, the data to be analyzed was in the form of a 12 × 31 element matrix (2 scans/replicates × 3 replicates/treatment × 2 treatments with 31 DF values). The critical values of DF intensity and CV were assessed using PCA, which could be potentially used for real-time detection of *B. cepacia* caused sour skin in onion bulbs using portable FAIMS. The data analysis was done in MATLAB® (Version R2016a, Mathworks, Natick, MA, U.S.) and statistical environment R (version 3.2.2, R Foundation for Statistical Computing, Vienna, Austria).

Results and discussion

FAIMS based sour skin detection in onion bulbs

Figure 2 shows representative DF matrices in positive mode (a, b), ion current plots at 60% DF intensity (c, d), and RGB images (e, f) for healthy control and inoculated onion bulbs respectively on 3 DAI. On 3 DAI, for all the replicates of inoculated treatment, sour skin pertinent VOCs were detected by FAIMS. Prior to 3 DAI (0–2 DAI), both the treatments reported similar response, indicating similar state of VOCs release. In the DF matrix for healthy controls (Fig. 2a), a prominent peak shifting towards left could be observed. It corresponds to reactant ion peak (RIP) formed by the ions of the carrier gas. The other peak moving up corresponds to the VOCs pertinent to healthy controls. VOCs are released even from healthy tubers, however, due to stress caused by infection, the type or quantity of the VOCs release may change [9] temporally. For sour skin infected onion bulbs (Fig. 2b), no RIP was observed. This disappearance of RIP could be attributed to utilization of reactant ions for carrying the sour skin associated VOCs to the FAIMS analyzer. Greater the VOCs released, feebler was the RIP. The ion current plots for healthy and inoculated treatments (Fig. 2c, d) showed a second peak corresponding to the VOCs associated with sour skin. The bulbs which were detected for infection by FAIMS, were visually same as compared to the healthy bulbs (Fig. 2e, f). Overall, the FAIMS based response of healthy and sour skin infected onion bulbs confirmed the suitability of portable FAIMS towards early detection of *B. cepacia* caused sour skin in stored onions.

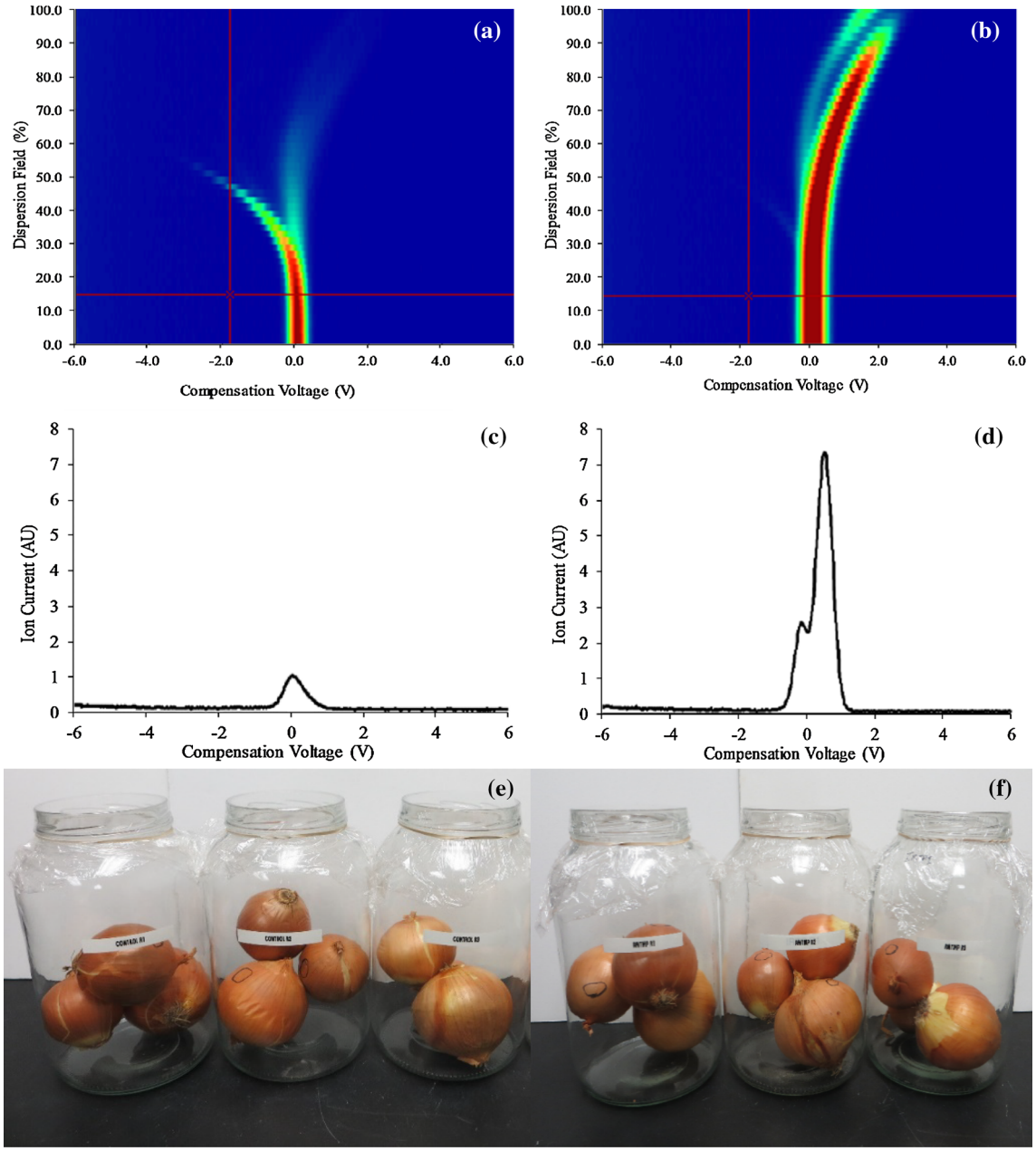

Fig. 2 FAIMS based response in terms of DF matrix (**a**, **b**), ion current plots (**c**, **d**) and RGB images (**e**, **f**) respectively for healthy (*left*) and *B. cepacia* inoculated onion bulbs (*right*)

Figure 3 shows the PCA score plots for healthy and *B. cepacia* inoculated onion bulbs on few representative DAIs. Each data point on the score plot represents the principal component scores of each of the treatment replicates. The first two principal components (PC1 and PC2) explained more than 99% of the variance in FAIMS based response for the VOCs collected in the headspace. Thus, the redundancy in the FAIMS based response of *B. cepacia* caused sour skin was effectively reduced using the first two principal components. Although the FAIMS based response of VOCs were captured on 10 different days in the experimental timeline, shown are some of the representative score plots. It is evident from the score plot for 0 DAI (Fig. 3a) that no clear distinction among the treatments was observed, indicating similar pattern of VOCs release. On 3 DAI (Fig. 3b), inoculated treatment formed distinct cluster compared to healthy controls. The clusters corresponding to controls were comparatively more compact compared to inoculated treatment. For inoculated treatment, different replicates underwent different degree of infection,

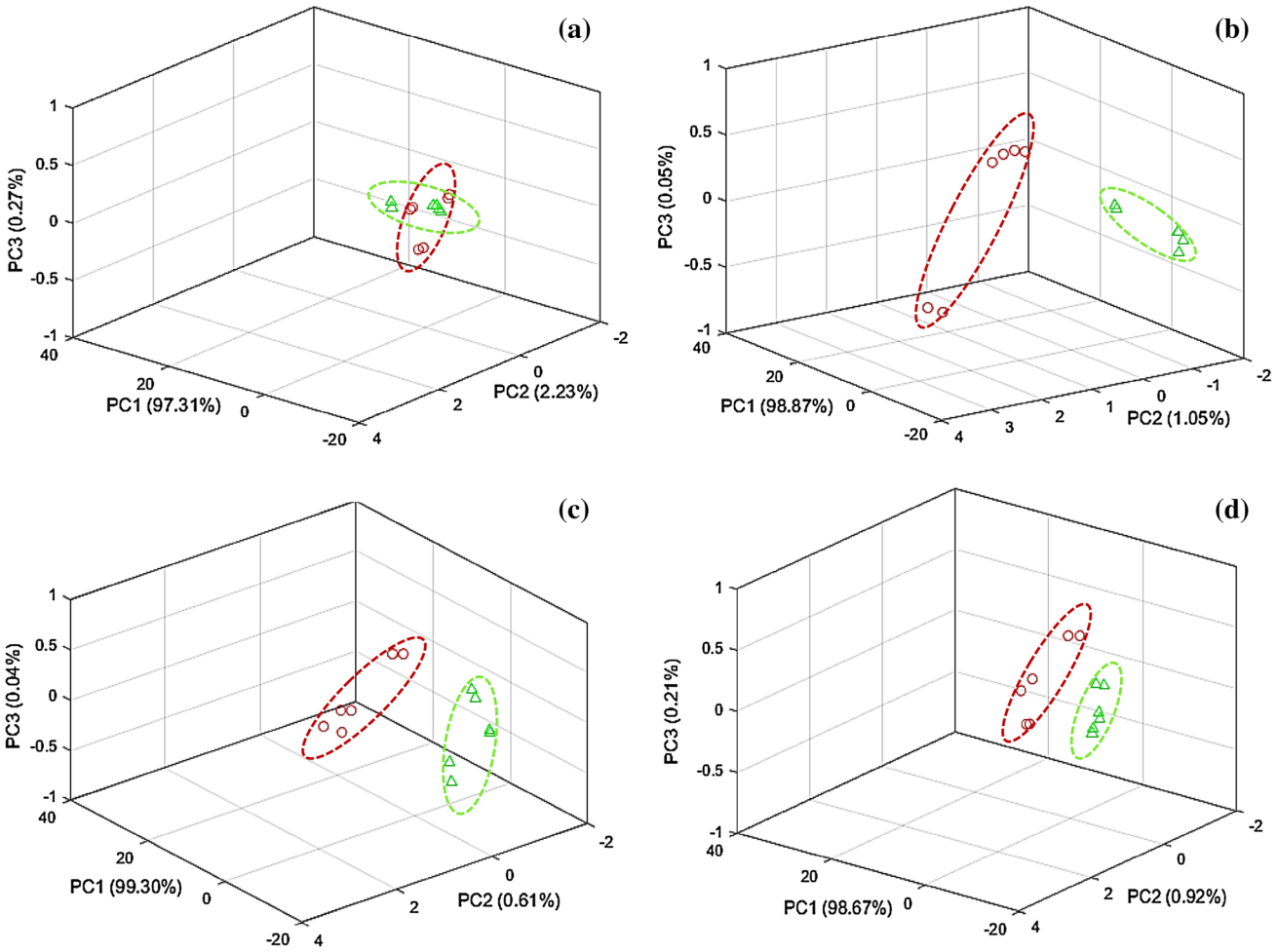

Fig. 3 FAIMS response based PCA score plots of healthy (*open triangle*) and sour skin infected (*open circle*) onion bulbs on **a** 0, **b** 3, **c** 6 and **d** 11 DAI

which could be attributed to factors like moisture content and prior bacterial colony present on the bulbs. This could possibly have caused non-uniform clusters for inoculated treatment. The cluster corresponding to inoculated treatment started to become small and compact at some later stages, i.e., on 11 DAI (Fig. 3d). This could possibly have been caused due to the reduced quantity of VOCs emission in the treatment replicates, causing a similar state of VOCs emission. It could further be observed that the separation between clusters was maximum on 3 DAI (Fig. 3b), that further reduced on 6 DAI (Fig. 3c). This indicates a reduction in the VOCs release on 6 DAI compared to 3 DAI. Li et al. [5] also reported that the abundance of VOCs in *B. cepacia* inoculated onions were 30 times higher than control healthy samples on 3 DAI. The abundance of VOCs on 6 DAI was reported to be 23 times higher than control.

Figure 4 shows the score plot for cumulative FAIMS response, where first two principal components (PCs) explained more than 99% of the variance in the data. There

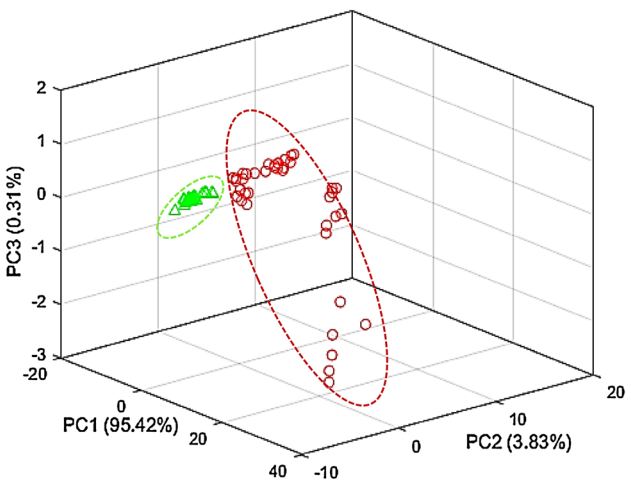

Fig. 4 FAIMS response based PCA of complete dataset showing class discrimination power of captured VOCs signatures [healthy (*open triangle*) and sour skin infected (*open circle*) onion bulbs]

existed a hyperplane which differentiated healthy controls from inoculated treatment. The cluster corresponding to healthy control was more uniform and compact throughout the study. This was expected as the healthy control released similar concentration of VOCs throughout the study period. The cluster corresponding to inoculated treatment was clearly separated from the cluster of healthy control. The cluster corresponding to infected samples had some sub-clusters. Li et al. [5] reported that different VOCs were released in different concentration corresponding to sour skin infection on different DAI. For some VOCs, the concentration increased, while for others, it decreased. This change in VOCs concentration could possibly be attributed to the non-uniform clustering and the presence of sub-clusters within the cluster of inoculated treatment.

FAIMS response to *B. cepacia* caused sour skin

The dataset was further analyzed using PCA to obtain the DF and CV range which significantly contributed to the first two PCs on each sampling day. This range of DF and CV could be critical in training the portable FAIMS for the real time sour skin monitoring and alarm facility operators if the VOCs pertinent to sour skin are present in the sampling headspace. To understand the FAIMS response, the obtained DF and corresponding CV were plotted as histograms which resolved to most significant range contributing to PCs. Most critical DF was between 44 and 77% with around 66% of the total calculated DF values existing in this range. For CV, the significant values ranged from −0.24 to 0.48 V. Fig. 5 shows the ion current plots of healthy and *B. cepacia* caused sour skin infected onion bulbs in the complete CV range (i.e., from −6 to 6 V), for two of the DF intensities (randomly selected) from the range identified through the analysis in previous sub-section. Compared to healthy control, a significantly higher ion currents for inoculated treatment were observed. This was further confirmed by comparing the mean ion current for healthy and inoculated treatments using paired t-test (at $\alpha = 0.05$) (Table 1). Overall, trends suggest a significant increase in the ion current from 3 DAI for the inoculated treatment, compared to the healthy control. This confirms the time frame of sour skin detection, as reported by the ion currents (Fig. 2d) and PC score plots (Fig. 3b). It could also be observed that for the inoculated treatment, the ion current increased from 3 DAI and reduced comparatively on 6 DAI. This could be attributed to differential release of sour skin specific VOCs on 3 and 6 DAI as reported by Li et al. [5]. Thus, significantly higher ion current in the ranges of 47–77% DF intensity and −0.24 to 0.48 V (CV) in positive DF matrix could be an indicator of *B. cepacia* caused sour skin. However, this range could be variety specific and further studies are warranted to test other bulk stored varieties.

Overall, FAIMS based volatile biomarkers sensing can be a promising technique to detect sour skin caused by *B. cepacia* in onion bulbs under bulk storage conditions. However, FAIMS does not undertake chemical analysis of the analytes present in the VOCs and hence, does not quantify what VOCs are being detected. The key VOCs pertinent to *B. cepacia* caused sour skin are sulfur compounds (dipropyl disulfide, propylpropenyl disulfide, methyl propyl disulfide etc.), and aliphatic compounds (2-undecanone, 2,4-octanedione, 3(2H)-furanone, 2-hexyl-5-methyl—etc.) [5]. Our ongoing studies are evaluating the response of FAIMS towards the above mentioned standard compounds as well as towards the volatile release variation and time

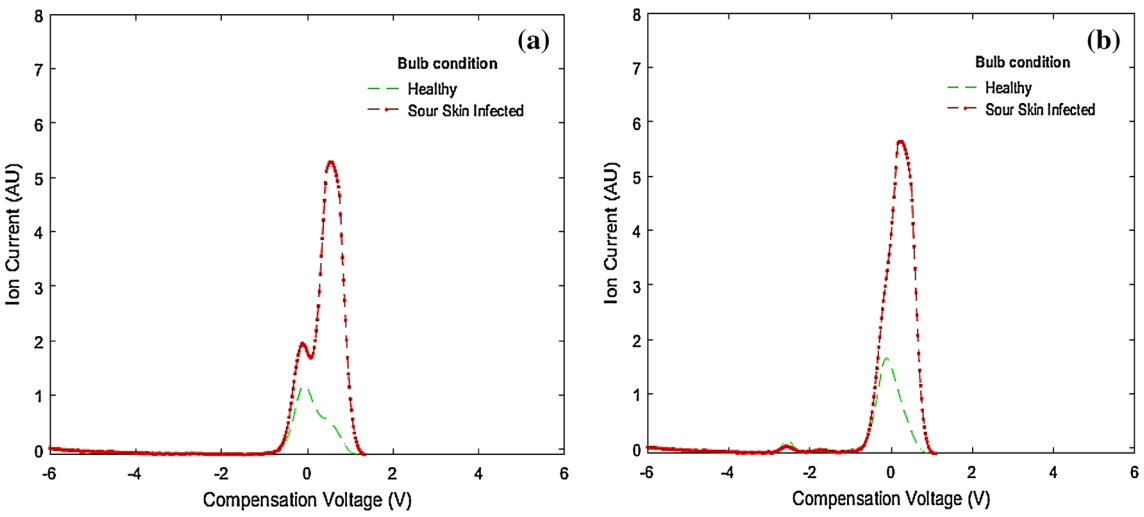

Fig. 5 Typical ion current from FAIMS response depicting healthy and *B. cepacia* caused sour skin associated peaks on 3 DAI for **a** 50 and **b** 60% DF intensity

Table 1 FAIMS response derived maximum ion current data depicting effectiveness of the approach in *B. cepacia* caused sour skin identification on different DAI(s) at selected DF intensities

DAI	Ion current (mean ± std. error), AU					
	60% DF			70% DF		
	Treatment-1	Treatment-2	p-value	Treatment-1	Treatment-2	p-value
0	2.10 ± 0.05	1.74 ± 0.06	0.001	1.05 ± 0.07	0.94 ± 0.05	0.729
1	1.52 ± 0.11	1.52 ± 0.14	0.986	0.91 ± 0.12	0.98 ± 0.12	0.732
2	1.34 ± 3.04	3.04 ± 0.93	0.099	0.94 ± 0.12	2.55 ± 0.88	0.098
3	1.18 ± 0.12	5.43 ± 1.43	0.014	0.84 ± 0.11	5.13 ± 1.52	0.018
4	0.85 ± 0.02	6.35 ± 1.41	0.003	0.56 ± 0.02	5.99 ± 1.49	0.004
5	0.50 ± 0.14	6.41 ± 0.52	<0.000	0.29 ± 0.08	5.84 ± 0.51	<0.000
6	0.92 ± 0.14	5.01 ± 0.70	<0.000	0.59 ± 0.13	4.47 ± 0.64	<0.000
11	0.95 ± 0.12	2.45 ± 0.29	<0.000	0.39 ± 0.09	1.93 ± 0.32	<0.000
16	0.63 ± 0.06	2.34 ± 0.31	<0.000	0.30 ± 0.05	1.69 ± 0.25	<0.000
21	0.81 ± 0.05	1.45 ± 0.21	<0.000	0.27 ± 0.03	0.92 ± 0.19	0.006

frame for detection related to varied bulk storage temperature–humidity conditions.

Real-time detection of *B. cepacia* caused sour skin VOCs can be highly instrumental for storage managers and growers in making management decisions for bulk stored produce. FAIMS is sought for such a purpose due to the portability option. Using the significant range of DF and CV, as reported in this study, it is possible to train the FAIMS to trigger an alarm for real-time and early sour skin detection. Once the occurrence of sour skin is alarmed by the system, rigorous inspection of the facility could be initiated to take quick management decisions for saving a large part of the produce. One possible approach might be to use an automated platform integrated with the portable FAIMS, to continuously sample VOCs inside the facility. This could easily locate the critical zones inside the storage facility of the sour skin infected onions. Our ongoing studies are also exploring the feasibility of infrared imaging to detect sour skin in onions under bulk storage conditions. Hypothetically, once the critical zones are located, they can be surgically removed, preventing further crop loss.

Conclusions

The feasibility of portable FAIMS was evaluated to detect *B. cepacia* caused sour skin in bulk stored onions. The release of VOCs from healthy controls and sour skin infected onion samples was monitored over a 21-day storage period. The portable FAIMS system was reported to detect sour skin and differentiate between healthy controls and the sour skin infected samples, within 3 days after inoculation. Such detection time frame was further confirmed by PCA and paired t-test on FAIMS based maximum ion current response. In terms of FAIMS training, and applicability in bulk storage for sour skin detection, results confirmed a range of significant DF (47–77%) and CV (−0.24 to 0.48 V). Our ongoing studies are focused on evaluating the response of portable FAIMS to sour skin under varied bulk storage temperature–humidity conditions and relating the response to specific biomarkers, by studying the FAIMS response towards key VOCs reported to be associated with sour skin.

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