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FAIMS based volatile fingerprinting for real-time postharvest storage infections detection in stored potatoes and onions

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ABSTRACT

Field asymmetric ion mobility spectrometry (FAIMS) was evaluated towards rapid and non-destructive detection of storage infections under varied storage conditions. Potato tubers and onion bulbs were inoculated with *P. carotovorum* subsp. *carotovorum* (causing soft rot) and *B. cepacia* (causing sour skin), respectively; and were incubated at room (around 25 °C) and reduced temperature condition (4 °C). Additional tubers and bulbs were inoculated with sterile water, which served as healthy controls. At room temperature, FAIMS could detect potato soft rot and onion sour skin pertinent volatile organic compounds (VOCs) as early as 1 and 3 day(s) after inoculation (DAI) for potato tubers and onion bulbs, respectively. At a reduced temperature (4 °C), the respective detection time frames were 11 and 16 DAI. Principal component analysis (PCA) based contribution analysis on FAIMS dispersion field data revealed a significant range of dispersion field (DF) intensity (52%–72%) and compensation voltage (CV) (−1.30 V to −0.90 V) that can potentially be used to train FAIMS for triggering an alarm during real-time monitoring of soft rot pertinent VOCs. This critical range was 47%–77% DF and −0.24 V to 0.48 V CV for sour skin pertinent VOCs. Naïve Bayes (NB) and linear discriminant analysis (LDA) classifiers tested on PCA datasets reported overall accuracies in the range of 71–100% and 69–100% for soft rot and 63–97% and 58–100% for sour skin, respectively. Higher accuracies were reported as days after inoculation progressed. Baseline sensing of different VOCs using FAIMS revealed that ethanol, acetone, 2-butanone and ethyl acetate were specifically contributing to *P. carotovorum* subsp. *carotovorum* caused soft rot peaks whereas pentane and 1-butanol were associated with healthy as well as inoculated tubers. Dimethyl disulfide, dipropyl disulfide, methyl propyl disulfide, undecane and 2-undecanone were found to be associated with healthy controls as well as with sour skin infected onion bulbs.

1. Introduction

Potatoes (*Solanum tuberosum*) and onions (*Allium cepa*) are two important storage crops in United States (US). A large part of the produce is stored for 9–12 months to meet year-round consumer demand. The recommended storage temperature is 4–7 °C for consumable produce of potatoes (Voss and Timm, 2016), with minimum respiration rate for storage at 5 °C (Potato Council, 2012). The recommended storage temperature for onions is between 1 and 7 °C (USA Onions, 2016). During bulk storage, the crops are susceptible to many bacterial and fungal diseases. Major potato storage diseases include soft rot (*Pectobacterium carotovorum* subsp. *carotovorum*), ring rot (*Clavibacter michiganesis* subsp. *sepedonicus*) and pink rot (*Phytophthora erythroseptica*) (Kushalappa et al., 2002). These storage diseases can cause an average loss of about 7.5% annually in the U.S. potatoes (Olsen et al., 2006).

The major postharvest issue for onions are storage rots, which may be caused by more than 26 pathogens encompassing bacteria, filamentous fungi, and a yeast. Different bacteria like *Burkholderia cepacia, Dickeya chrysanthemi, Enterobacter cloacae* and *Pectobacterium carotovorum* subsp. *carotovorum* are documented to cause storage rots in onions. Sour skin infection in onions usually propagates in the field. However, losses mostly appear under storage causing a yield loss of 5–50% (Schwartz and Mohan, 2008). Bulk of the produce is wasted due to diseases in both the storage crops and losses can be as high as 100% for individual storage facilities (Pelter and Sorensen, 2004). Considering the economic losses to growers due to storage infections; it is imperative to detect such infections as early as possible. Early detection can lead towards better management practices being initiated to minimize the losses in these crops under storage conditions.

Produce under storage (e.g. potato, onion and carrot) naturally

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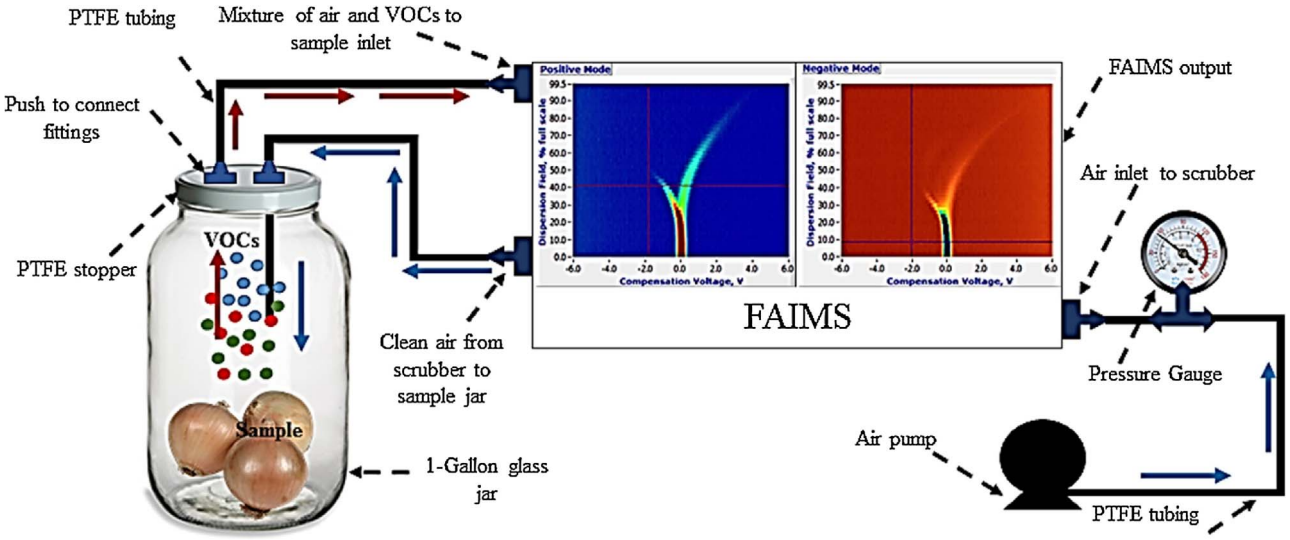

Fig. 1. Schematic for VOCs analysis using a customized module integrated with the portable FAIMS.

produce volatiles, which increase with disease severity, and physical or freeze damage (Toivonen, 1997; Jansen et al., 2011). Efforts towards detecting disease or stress specific volatile organic compounds (VOCs) from produce under storage were completed using gas chromatography mass spectrometry (GC–MS), GC–flame ionization detector (FID) and electronic nose (e–nose) (Varns and Glyn, 1979; Jarvenpaa et al., 1998; Kjeldsen et al., 2003; Prithiviraj et al., 2004; Li et al., 2011; Biondi et al., 2014; Konduru et al., 2015; Rutolo et al., 2016). GC–FID technique was used to discriminate potato tubers (incubated at 20 °C) inoculated with different bacteria (Kushalappa et al., 2002). Rutolo et al. (2016) developed an array of metal–oxide based gas sensors to detect *P. carotovorum* subsp. *carotovorum* related soft rot in potato tubers. The tubers, after inoculation, were kept in sealed plastic boxes and were incubated at 25 °C. de Lacy Costello et al. (1999) used GC–MS technique to identify the VOCs generated by potato tubers inoculated with *E. carotovorum* (now *P. carotovorum* subsp. *carotovorum*), *Bacillus polymyxa and Arthrobacter* sp. incubated at 10 °C. Li et al. (2011) used a gas sensor array to detect Botrytis neck rot (bulbs incubated at 24 ± 2 [mean ± std. dev.] °C) and sour skin (bulbs incubated at 30 °C) in onions, followed by VOCs quantification through GC–MS.

Recently, portable field asymmetric ion mobility spectrometry (FAIMS) was also used to detect huanglongbing disease in citrus (Alexander et al., 2014) and infections in stored potatoes and onions incubated at room temperature (25 ± 1 [mean ± std. dev.] °C) (Rutolo et al., 2014; Sinha et al., 2017a,b). Rutolo et al. (2014), using the potato variety Maris Piper, reported that differences between healthy and inoculated samples were observed in both positive and negative ion matrices of FAIMS. However, Sinha et al. (2017a), using the potato variety Russet Burbank, reported differences to be observed in only the positive ion matrix of FAIMS. FAIMS based biomarkers were studied to detect VOCs pertinent to sour skin infection in onions stored at room temperature (around 25 °C) (Sinha et al., 2017b). It is evident from these studies that most research to detect storage infections have been carried out at room temperature, which is very different from the bulk storage conditions (reduced temperature of 4 to 7 °C). Moreover, very few studies have evaluated FAIMS for disease detection of stored produce, and no study has reported the applicability of FAIMS for detection of postharvest storage disease in onions under reduced temperature condition.

Therefore, overall goal of the study was to study the pattern of release of VOCs from potatoes and onions, inoculated with *P. carotovorum* subsp. *carotovorum* and *B. cepacia* causing soft rot and sour skin respectively, stored under their respective bulk storage temperature conditions using portable FAIMS. The FAIMS based response for the detection of soft rot in potatoes and sour skin in onions were evaluated under room temperature conditions T1 (around 25 °C) and reduced temperature condition T2 (4 °C). Storing the samples was logistically more convenient at T2 and close to the temperature of minimum respiration rate, i.e. 5 °C, of the stored produce. The specific objectives were to evaluate the applicability of FAIMS towards: 1) detection of *P. carotovorum* subsp. *carotovorum* caused soft rot and *B. cepacia* caused sour skin in potatoes and onions respectively under bulk storage conditions, 2) assessment of the detection time frame of storage infections under bulk storage conditions, and 3) characterize FAIMS response for rapid disease onset monitoring and contrast it with infestation related volatile biomarkers.

2. Materials and methods

2.1. Sample preparation and inoculation

P. carotovorum subsp. *carotovorum* strain Ec101 inoculum was prepared as described previously (Sinha et al., 2017a). *Burkholderia cepacia* strain BsWSU1 inoculum was prepared as previously described (Schroeder et al., 2012). Potato tubers (*Solanum tuberosum* cv. Burbank) and onion bulbs (*Allium cepa* cv. Vaquero) were inoculated with *P. carotovorum* subsp. *carotovorum* strain Ec101 and *B. cepacia* strain BsWSU1, respectively. Potato tubers and onion bulbs were also inoculated with sterile water as healthy controls. Potato tubers were obtained from the produce of 2015, which were stored at a commercial storage facility (AgriNorthwest Inc., Prescott, WA). Tuber inoculations with *P. carotovorum* subsp. *carotovorum* strain Ec101 were completed as described previously (Sinha et al., 2017a). Yellow storage onion bulbs from the produce of 2015 (cv. Vaquero) were procured from a local grocery store. The dry skins of onion bulbs with no visible bruises or damage were removed from the bulbs. *B. cepacia* strain BcWSU1 was grown overnight in a 5 ml NBY at 28 °C with agitation, inoculum standardized to 1×10^6 CFU/ml and injected into onion bulbs as previously reported (Schroeder et al., 2012). Tubers and onion bulbs were inoculated with sterile water to serve as controls.

2.2. Experimental module

VOCs released from inoculated samples, and the healthy controls were sampled using a custom sampling module (Fig. 1). The glass jar sealed on top with Polytetrafluoroethylene (PTFE) stopper facilitated the accumulation of VOCs in the headspace. The inlet and outlet ports on the stopper were used to circulate the accumulated VOCs for

analysis. The detailed working of the experimental module has been reported in Sinha et al. (2017a). In the potato study, sterile water (50 ml) was put in a petri dish at the bottom of each jar to keep the environment inside the jars humid to facilitate the progression of rot. Samples were misted with sterile water on each sampling day after headspace sampling was completed to facilitate soft rot progression (Rutolo et al., 2014; Sinha et al., 2017a).

2.3. Data acquisition procedure

The monitoring and subsequent sampling of VOCs released from inoculated and healthy treatments of both potatoes and onions was accomplished in two phases. In phase-1, VOCs were sampled on 0, 1, 2, 3, 4, 5 and 6 days after inoculation (DAI), where 0 DAI stands for VOCs sampling time right after inoculation. Sinha et al. (2017a,b) reported that the VOCs release pertinent to storage infections was highest in second week (for potato soft rot) and first week (onion sour skin) after inoculation, and the release of disease specific VOCs tended to respectively saturate in the third week and second week after inoculation. Thus, in phase-2, sampling was carried out every five days after the first week until 21 DAI (i.e. 11, 16 and 21 DAI). Treatments incubated at a reduced temperature, i.e. T2 (TP2 and TO2), were sampled for VOCs after jars were moved to room temperature for an hour. On each sampling day, for each replicate, the VOCs in the headspace of the sampled jars were scanned 6 times. A system blank, with air only, was run (around 15 min) after every sampling to purge the FAIMS column which checked cross contamination from the previous samples. The detailed working of the integrated module and data acquisition protocol are in Sinha et al. (2017a).

2.4. Experimental design

The experimental design included three treatments per crop (two inoculated with pathogen and one inoculated with sterile water) with four replicates of either of potato tubers (in case of TP1, TP2 and TP3) or onion bulbs (in case of TO1, TO2 and TO3). In both the studies, treatment 1 (TP1 and TO1 for potatoes and onions respectively) consisted of samples inoculated with pathogen (*P. carotovorum* subsp. *carotovorum* for potatoes and *B. cepacia* for onions) and incubated at room temperature (25 °C). Treatment 2 (TP2 and TO2) consisted of samples inoculated with pathogen and incubated at reduced temperature (T2, i.e. 4 °C). Treatment 3 (TP3 and TO3) consisted of samples inoculated with sterile water and incubated at room temperature (T1, i.e. 25 °C). These were used to characterize the resulting FAIMS based detection for soft rot and sour skin under room and reduced temperature conditions compared to the healthy controls. Summary of various treatments under study and experimental details is given in Table 1.

2.5. FAIMS datasets

The portable FAIMS analyzer outputs positive as well as negative ion current plots, also called as dispersion field (DF) matrices. Each DF matrix represents a 3D matrix of ion current values corresponding to changing DF intensity and compensation voltage (CV). The DF intensity changes from 0 to 100% in 51 steps and CV changes from −6 to 6 V in 512 steps, generating a matrix of 51 × 512 ion current values (Parris et al., 2014). Preliminary evaluation of FAIMS based response for *P. carotovorum* subsp. *carotovorum* caused soft rot in stored potatoes revealed that the differences among treatments were observed in the DF range of 30%–90%. In the onion assay, this range was 40%–100%. Maximum ion current values corresponding to these DF intensities were extracted. Thus, in the tuber soft rot study, for each sampling day, the data to be analyzed was in the form of 24 × 31 element matrix (3 treatments × 4 replicates per treatment × 2 scans per replicates with 31 maximum ion current values corresponding to each selected DF% and CV values). For the complete tuber study, the data set to be analyzed was a 240 × 31 element matrix (24 scans per sampling day × 10 sampling days). Similarly, in the onion sour skin study, for each sampling day, the data to be analyzed was in the form of 24 × 31 element matrix (3 treatments × 4 replicates per treatment × 2 scans per replicates with 31 maximum ion current values).

2.6. Data analysis

Six DF scans were acquired for each replicate on each sampling day. The ion current intensity increased initially, then stabilized and finally decreased in order of DF scans 1–6. Two of the six scans, where the ion current was stable, were used for data analysis. The data analysis protocol was as reported in Sinha et al. (2017a). The FAIMS based response for soft rot in potatoes and sour skin in onions was analyzed using Principal Component Analysis (PCA) (Jolliffe, 2002). Naïve Bayes (NB) and Linear Discriminant Analysis (LDA) classifiers were used to classify the healthy and inoculated treatments with 10–fold cross validation. An NB classifier assigns a probability value to a given class based on conditional independence assumption among the features and converges quickly to pertinent asymptotic error (Ng and Jordan, 2002). The LDA utilizes pooled co-variance in Bayes' criteria for assigning a random sample to a specific class (Naes et al., 2002) and has been used to classify healthy and infected crop samples (Singh et al., 2012; Sankaran et al., 2013; Rutolo et al., 2014). Classification of healthy and inoculated treatments was accomplished using the first three principal components (PC1, PC2 and PC3) from PCA as input features. Confusion matrix was used as a metric for testing the quality of developed classification model. In the next phase of data analysis, the critical values of DF intensity and CV were assessed using PCA. These critical DF and CV values could potentially be used for real-time detection of soft rot and sour skin in potatoes and onions respectively, stored under bulk storage conditions. The data analysis was performed in MATLAB® (Version R2016a, Mathworks, Natick, MA, U.S.) and statistical environment R (version 3.2.2, R Foundation for Statistical Computing, Vienna, Austria) using methods described previously (Sinha et al., 2017a).

2.7. Relationship between FAIMS ion peaks and VOCs

Relationship between FAIMS based ion peaks with associated volatile biomarkers was established by testing key VOCs reported in prior studies pertinent to *P. carotovorum* subsp. *carotovorum* caused soft rot and *B. cepacia* caused sour skin as standards. Key VOCs associated with

Table 1
Description of various treatments and experimental details used in the study.

Crop	Treatment	Inoculation Type	No. of Replicates	Storage Temperature (°C)	No. of scans	No. of DF Intensity	No. of sampling days
Potato	TP1	*P. carotovorum*	4	25	2	51	10
	TP2	*P. carotovorum*	4	4	2	51	10
	TP3	Sterile water	4	25	2	51	10
Onion	TO1	*B. cepacia*	4	25	2	51	10
	TO2	*B. cepacia*	4	4	2	51	10
	TO3	Sterile water	4	25	2	51	10

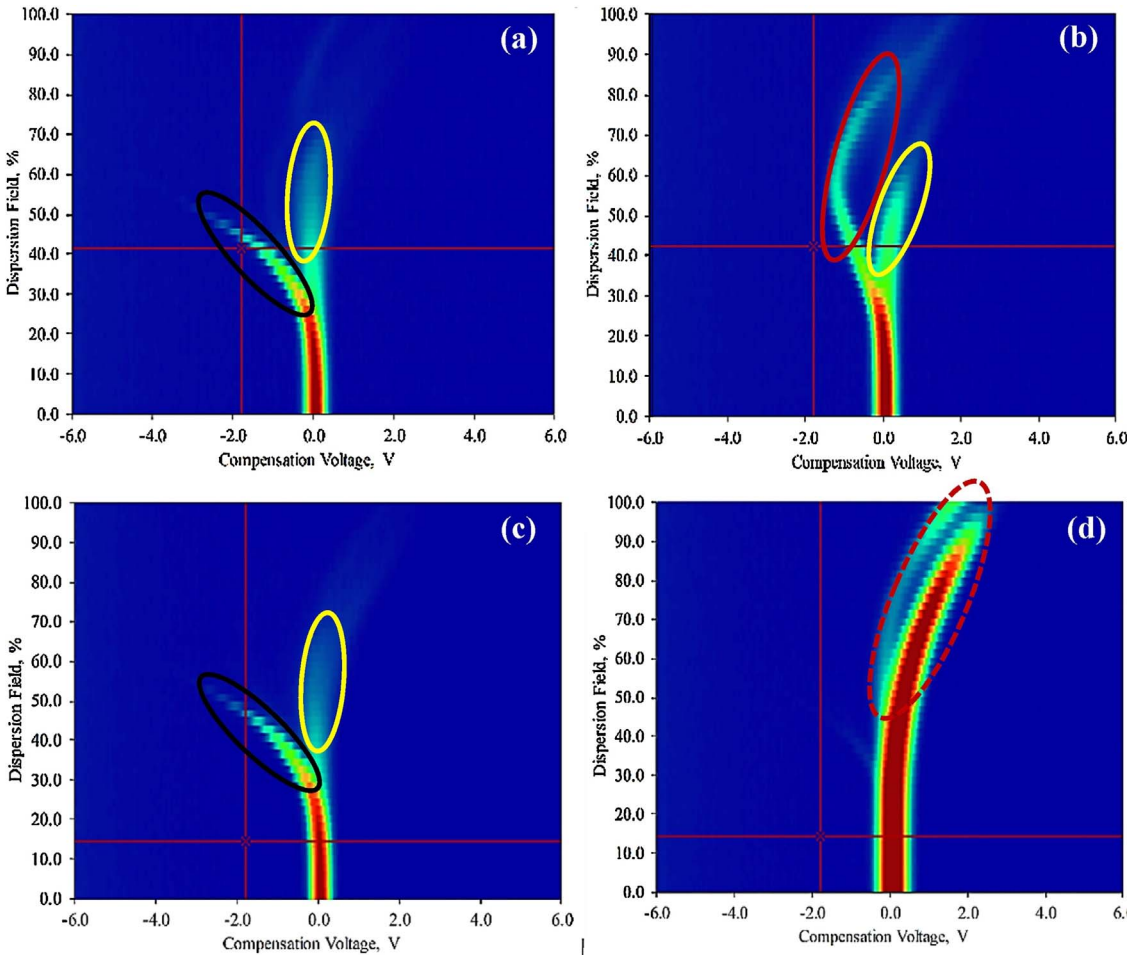

Fig. 2. Representative FAIMS derived positive DF matrices for healthy and infected treatments respectively for soft rot in potatoes (a, b) and sour skin in onions (c, d) (Highlighted peaks with elliptical shapes represent RIP [black], VOCs common to both healthy and inoculated treatments [yellow] and VOCs pertinent to potato soft rot [red] and onion sour skin [red dashed]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

soft rot are reported to be acetone, ethanol, 2-butanone, 2-pentanone, acetaldehyde, acetic acid, pentane, and 1-butanol (Varns and Glyn, 1979; Waterer and Pritchard, 1984; de Lacy Costello et al., 1999). The key VOCs for sour skin in onions were reported to be undecane, dipropyl disulfide (DPDS), dimethyl disulfide (DMDS), propyl propenyl disulfide, 2-undecanone, methyl propyl disulfide (MPDS), methyl propenyl disulfide, 2,4-Octanedione, and 2-nonanone (Li et al., 2011). Key VOC standards were procured (Sigma Aldrich, St. Louis, Missouri, USA) and analyzed using FAIMS at two different concentrations by sampling 5 and 10 µl of the pertinent VOC standard in air. The protocol used to sample and analyze the VOCs pertinent to soft rot and sour skin was followed herein. Biomarker VOCs concentrations were generated following the methodology reported by Konduru et al. (2015) and pertinent results are presented.

3. Results and discussion

3.1. FAIMS response to storage infections

Fig. 2 presents typical FAIMS positive DF matrices for the healthy and inoculated treatments for potatoes exhibiting soft rot (Fig. 2a, b), and onions exhibiting sour skin (Fig. 2c, d) symptoms. Two major peaks were visible in the healthy controls in both the crops (Fig. 2a, c). The peak curving towards left (highlighted with black ellipse) composed of reactant ions from carrier gas, also known as reactant ion peak (RIP) (Parris et al., 2014). An entirely different peak (highlighted with solid red ellipse) (Fig. 2b) was observed because of potato soft rot infection. However, in the onion bulb rot assay, the intensity of an existing peak (highlighted with yellow ellipse) in healthy controls (Fig. 2c) increased due to sour skin infection (Fig. 2d). Therefore, it can be inferred that in potatoes, some VOCs exclusive to soft rot infection were released, and for onions, a similar set of VOCs were released in higher intensity, compared to healthy controls. Furthermore, the RIP disappeared in infected samples as reactant ions were utilized in carrying VOCs pertinent to storage infections to the FAIMS core. Prior studies (Varns and Glynn, 1979; de Lacy Costello et al., 1999; Li et al., 2011) have also reported that some of the VOCs are common to both healthy and infected treatments (yellow ellipse in Fig. 2a, b), while some are exclusively associated with the infection (red ellipse in Fig. 2b, d).

The VOCs pertinent to *P. carotovorum* subsp. *carotovorum* exhibiting soft rot in potatoes under storage were detected on 1 and 11 DAI for treatments at the room temperature (TP1) and at a reduced temperature (TP2), respectively. Sinha et al. (2017a) also reported the soft rot detection time frame at room temperature was as early as 1 DAI in one of the replicates. However, VOCs pertinent to *B. cepacia* exhibiting sour skin in onions were detected on 3 and 16 DAI for treatments at the room temperature (TO1) and at a reduced temperature (TO2), respectively. This was in line with the onion sour skin detection time frame reported by Sinha et al. (2017b). On these days, higher mean ion current values were recorded for inoculated tubers and onion bulbs compared to the control. The mean ion current values for all the treatments are plotted for soft rot of potatoes and sour skin of onions (Fig. 3). Ion current plots of the different treatments are shown only for those days, which were critical to the time frame of storage infection detection. The ion current

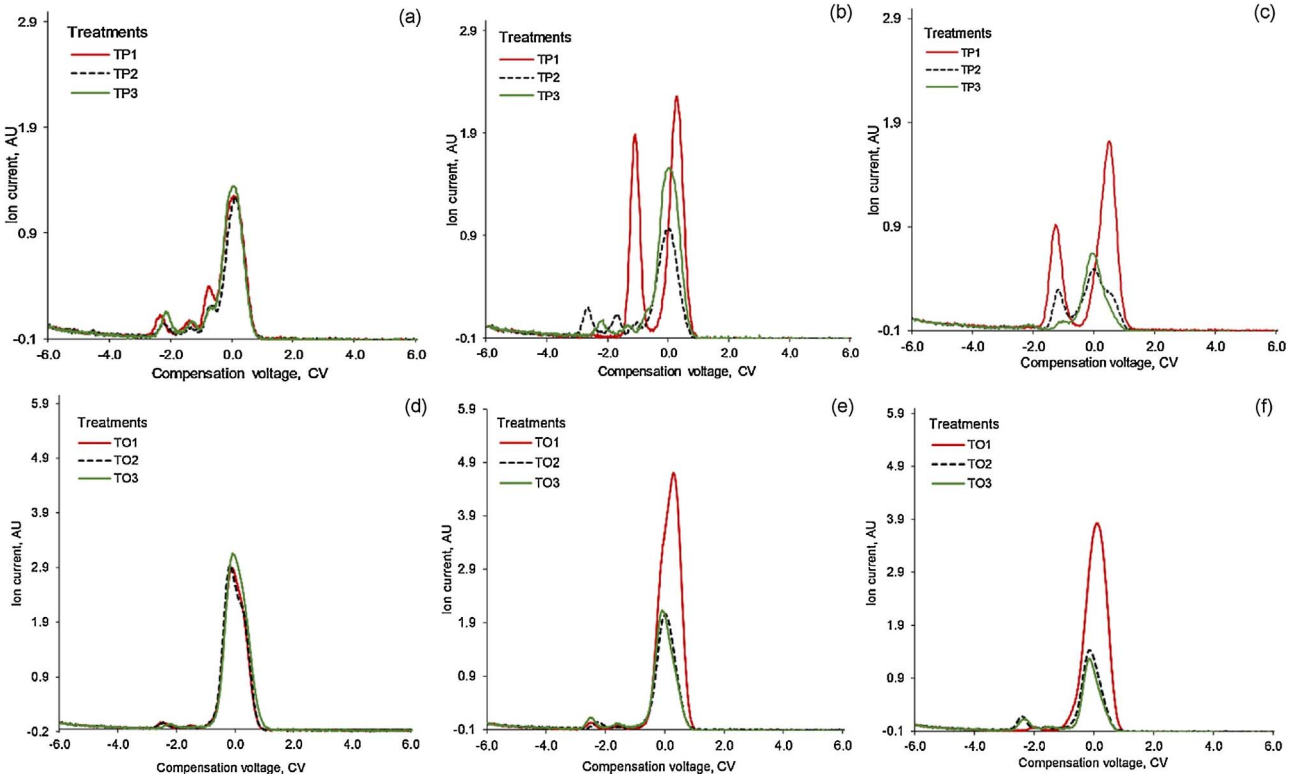

Fig. 3. FAIMS derived ion current plots corresponding to different treatments for *P. carotovorum* subsp. *carotovorum* inoculated potato tubers causing soft rot symptoms on a) 0, b) 1 and c) 11 DAI and for *B. cepacia* inoculated onion bulbs causing sour skin bulb rot on d) 0, e) 3 and c) 16 DAI.

plots for tubers inoculated with *P. carotovorum* subsp. *carotovorum* exhibiting soft rot in potatoes, and *B. cepacia* inoculated onion bulbs exhibiting sour skin in onions, as well as the water inoculated controls were identical on 0 DAI (Fig. 3a, d). However, on 1 and 3 DAI, a higher mean ion current value was reported for tubers exhibiting soft rot (Fig. 3b) and onion bulbs exhibiting sour skin (Fig. 3e). At reduced temperature (T2), VOCs indicative to soft rot and sour skin were detected on 11 DAI (Fig. 3c) and 16 DAI (Fig. 3f), respectively. This was expected as the activity of bacterial pathogens were checked under reduced temperature.

PCA was used to detect the coherent patterns in the FAIMS based response as different clusters (Fig. 4). The individual points in each of the clusters represent the PC scores corresponding to each replicate under different treatments. PC score plots of all the treatments on days critical to the time frame of soft rot and sour skin detection confirmed the temporal detection of potato soft rot (1 and 11 DAI, respectively at T1 and T2) and onion sour skin (3 and 16 DAI, respectively at T1 and T2). The first three PCs in potato soft rot study accounted for around 96% of the variance in the dataset, whereas, for onion sour skin, first two principal components (PCs) accounted for around 98% of the variance in the dataset (Fig. 4). Water controls in both the storage infections formed relatively smaller clusters, compared to inoculated treatments kept at room temperature. The clusters for inoculated treatments were non-uniform indicating different degree of pathogenic activity in different replicates by *P. carotovorum* subsp. *carotovorum* in potato and *B. cepacia* in onions. Pathogenic activity was quantified in terms of VOCs release as sample replicates were not analyzed for degree of pathogenic infection. For soft rot in potatoes, the clusters corresponding to treatment 2 (TP2) were compacted to start with (0–5 DAI), but the clusters started to grow large since 11 DAI, indicating the progression of rot under the reduced temperature (4 °C) (Fig. 4c). However, for onion sour skin, the cluster corresponding to TO2 were compact throughout the study (except 0 DAI), indicating decreased VOCs emission under reduced temperature condition (4 °C) (Fig. 4d–f).

3.2. Temporal progression monitoring and FAIMS tuning for infection detection

FAIMS based response of potato tubers inoculated with *P. carotovorum* subsp. *carotovorum* and *B. cepacia* inoculated onion bulbs were further analyzed using PCA to obtain the DF% and CV range which significantly contributed to the first two principal components (PCs) on each sampling day. This range of DF% and CV values will be critical in training the portable FAIMS for the real-time monitoring of VOCs pertinent to soft rot and sour skin development in storage and the subsequent notification of infected facility. DF range of 52%–72%, and CV range of −1.3 V to −0.9 V was found to be critical for VOCs pertinent to potato sour skin progression. Moreover, a DF range of 44%–77% and a CV range of −0.24 to 0.48 V was critical for VOCs related with onion sour skin progression. This could be observed in the mean ion current plots for soft rot and sour skin (Fig. 3), where values are plotted at 60% and 50% DF, respectively. Mean ion current for all the treatments in soft rot and sour skin were compared using analysis of variance (ANOVA) for two randomly selected DF intensities (58% and 68%) from the critical range (Tables 2 and 3). Overall, trends suggested a significant increase in the mean ion current for inoculated treatments, compared to the healthy controls. Thus, significantly higher ion current in the abovementioned range in positive DF matrix could be an indicator of *P. carotovorum* subsp. *carotovorum* infected potato tubers, and *B. cepacia* infected onion bulbs. However, such range could be variety specific and further studies are warranted to test other bulk-stored varieties.

3.3. Relating VOCs to FAIMS response

The ion current for key VOCs pertinent to *P. carotovorum* subsp. *carotovorum* caused potato soft rot (acetone, ethanol, 1-butanol, 2-butanone and ethyl acetate) and *B. cepacia* caused onion sour skin (DMDS, DPDS, MPDS, 2-undecanone and undecane) were compared with

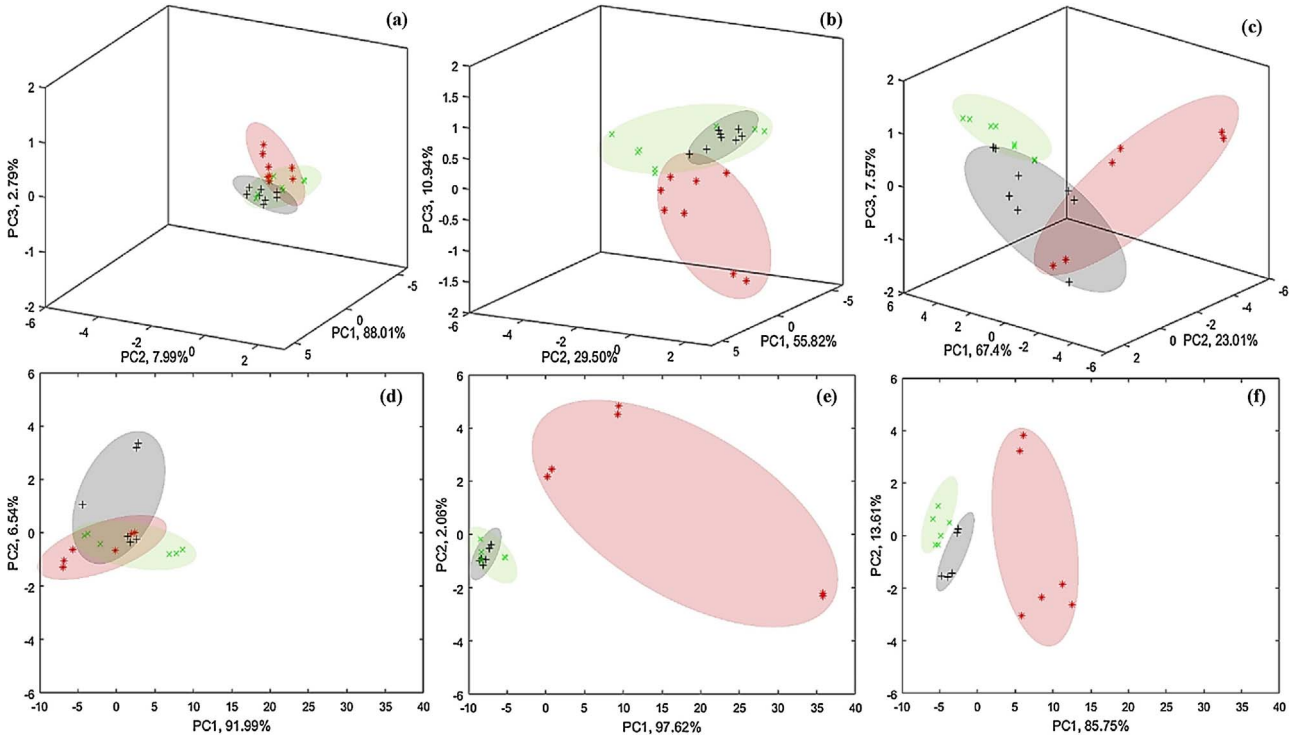

Fig. 4. FAIMS response based PC score plots for visualization of coherent patterns corresponding to different treatments [TP1 or TO1 (*); TP2 or TO2 (o); and TP3 or TO3 (×)] in potato soft rot on a) 0, b) 1, and c) 11 DAI and onion sour skin on d) 0, e) 3 and f) 16 DAI.

Table 2
FAIMS response derived mean ion current at two DF intensities (selected randomly from the critical range), for different treatments under study for detection of *P. carotovorum* subsp. *carotovorum* infected potato tubers.

DAI	Ion Current (Mean ± Std. Error), AU							
	58%				68%			
	TP1	TP2	TP3	*p*–value	TP1	TP2	TP3	*p*–value
0	0.27 ± 0.03^{a}	0.22 ± 0.03^{a}	0.23 ± 0.02^{a}	0.2200	0.17 ± 0.02^{a}	0.11 ± 0.01^{a,b}	0.05 ± 0.01^{b}	0.0004
1	1.32 ± 0.17^{a}	1.27 ± 0.06^{a}	0.24 ± 0.03^{a}	0.0006	0.60 ± 0.07^{a}	0.36 ± 0.01^{b}	0.06 ± 0.01^{c}	< 0.0001
3	1.14 ± 0.12^{a,b}	0.86 ± 0.19^{b}	0.76 ± 0.17^{b}	0.0060	0.99 ± 0.10^{a}	0.49 ± 0.22^{b}	0.13 ± 0.03^{b}	0.0005
4	1.18 ± 0.11^{a}	0.39 ± 0.01^{b}	0.63 ± 0.15^{b}	0.0020	1.02 ± 0.09^{a}	0.11 ± 0.01^{b}	0.13 ± 0.03^{b}	< 0.0001
5	1.21 ± 0.06^{a}	0.41 ± 0.02^{b}	0.61 ± 0.12^{b}	0.0001	1.04 ± 0.05^{a}	0.15 ± 0.01^{b}	0.19 ± 0.03^{b}	< 0.0001
6	1.46 ± 0.05^{a}	0.21 ± 0.04^{c}	0.47 ± 0.09^{b}	< 0.0001	1.25 ± 0.04^{a}	0.10 ± 0.01^{b}	0.12 ± 0.02^{b}	< 0.0001
11	1.22 ± 0.08^{a}	0.53 ± 0.13^{b}	0.34 ± 0.04^{b}	< 0.0001	0.91 ± 0.10^{a}	0.42 ± 0.12^{b}	0.06 ± 0.01^{c}	< 0.0001
16	1.77 ± 0.15^{a}	0.85 ± 0.17^{b}	0.19 ± 0.02^{c}	< 0.0001	1.18 ± 0.11^{a}	0.69 ± 0.15^{b}	0.05 ± 0.01^{c}	< 0.0001

Different letters represent significantly different ion current among different treatments on a particular DAI at α = 0.05.

Table 3
FAIMS response derived mean ion current values at two DF intensities (randomly selected from the critical range), for different treatments under study for detection of *B. cepacia* infected onion bulbs.

DAI	Ion Current (Mean ± Std. Error), AU							
	58% DF				68% DF			
	TO1	TO2	TO3	*p*–value	TO1	TO2	TO3	*p*–value
0	2.12 ± 0.07^{b}	1.96 ± 0.07^{b,c}	2.61 ± 0.09^{a}	< 0.000	0.95 ± 0.13^{a}	0.93 ± 0.09^{a}	1.05 ± 0.19^{a}	0.298
2	2.78 ± 1.07^{a}	2.50 ± 0.50^{a}	1.48 ± 0.15^{a}	0.395	2.55 ± 2.15^{a}	0.63 ± 0.21^{b}	0.94 ± 0.29^{a,b}	0.039
3	5.43 ± 1.43^{a}	1.06 ± 0.05^{b}	1.19 ± 0.12^{b}	0.003	5.12 ± 3.74^{a}	0.71 ± 0.04^{b}	0.85 ± 0.27^{b}	0.004
4	6.35 ± 1.42^{a}	0.97 ± 0.05^{b}	0.85 ± 0.03^{b}	< 0.000	7.57 ± 1.77^{a}	0.62 ± 0.10^{b}	0.56 ± 0.04^{b}	< 0.000
5	6.40 ± 0.53^{a}	1.13 ± 0.05^{b}	0.85 ± 0.24^{b}	< 0.000	5.84 ± 1.25^{a}	0.72 ± 0.10^{b}	0.29 ± 0.21^{b}	< 0.000
6	5.01 ± 0.70^{a}	0.89 ± 0.05^{b}	0.92 ± 0.14^{b}	< 0.000	4.47 ± 1.57^{a}	0.58 ± 0.10^{b}	0.59 ± 0.33^{b}	< 0.000
11	2.46 ± 0.29^{a}	0.90 ± 0.02^{b}	0.95 ± ag.12^{b}	< 0.000	1.93 ± 0.78^{a}	0.51 ± 0.02^{b}	0.39 ± 0.22^{b}	< 0.000
16	2.35 ± 0.31^{a}	0.97 ± 0.03^{b}	0.63 ± 0.06^{b}	< 0.000	1.69 ± 0.62^{a}	0.57 ± 0.04^{b}	0.30 ± 0.12^{b}	0.002

Different letters represent significantly different ion current among different treatments on a particular DAI at α = 0.05.

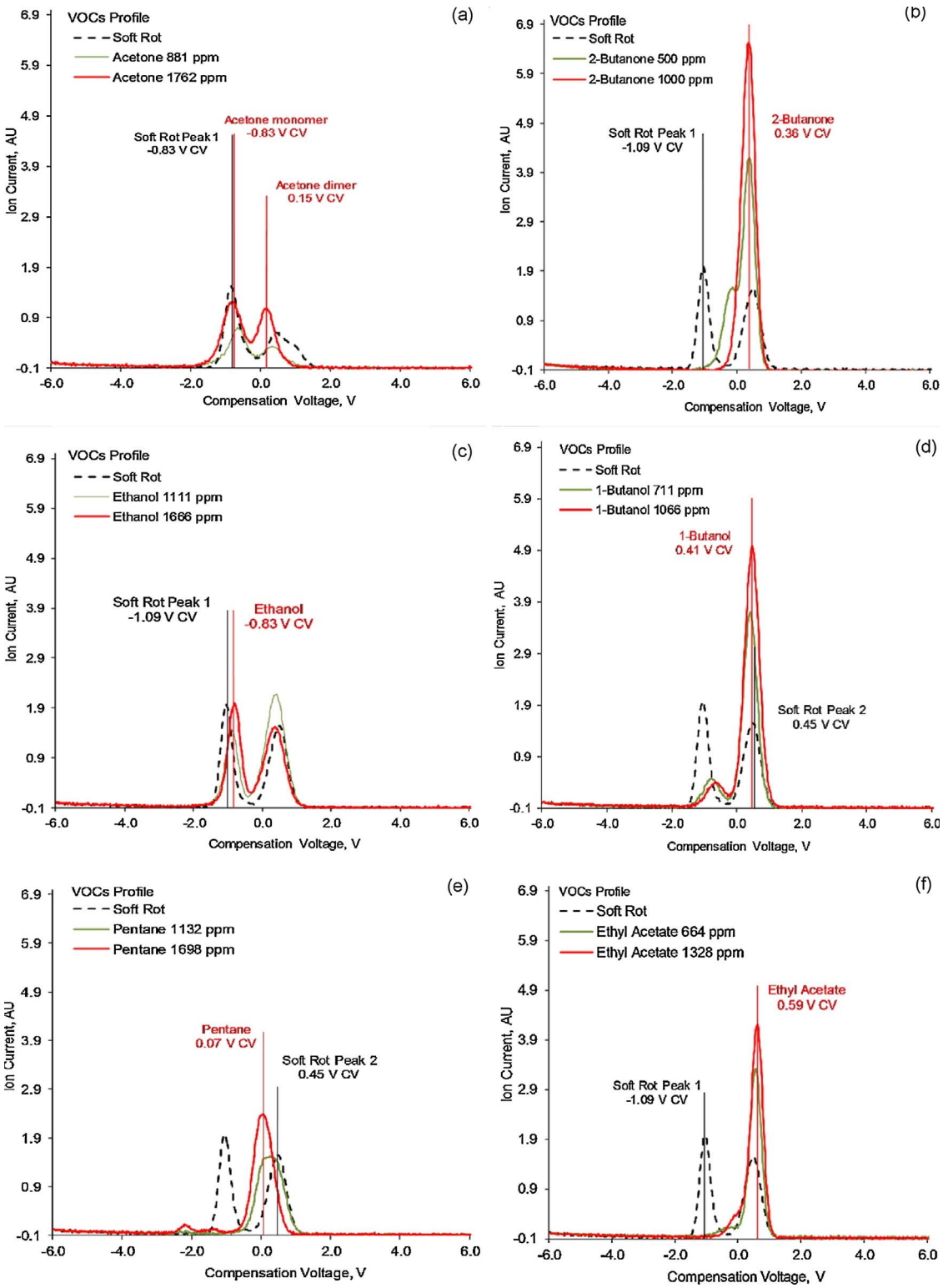

Fig. 5. FAIMS derived ion current plots for analytes a) acetone, b) 2-butanone, c) ethanol, d) 1-butanol, e) pentane and f) ethyl acetate at two different concentrations (the ion current plot of VOCs pertinent to onion sour skin is used as a baseline).

corresponding biological sampled ion current data (Figs. 5 and 6). FAIMS could distinguish different concentrations of all the analytes under investigation. Ion current peaks, like the ones found with VOCs pertinent to soft rot, were observed at very close CV for acetone (−0.83 V) (Fig. 5a), at a DF intensity of 70%. Another peak found in acetone plot could be an acetone dimer, as with a higher proton affinity and mass/charge ratio, two acetone monomers could be added to form dimers (Eiceman et al., 2002; Parris et al., 2014) which would peak at a higher CV. The DF matrices of 2-butanone and VOCs pertinent to potato soft rot exhibited similar characteristic curves. However, the ion peak for 2-butanone (Fig. 5b) was observed at 0.36 V compared to the soft rot peak at −1.09 V. The 2-butanone sample had a higher humidity (20.41%) compared to VOCs pertinent to potato soft rot (11.64%) which caused the 2-butanone peak to occur at higher CV, i.e. shifting

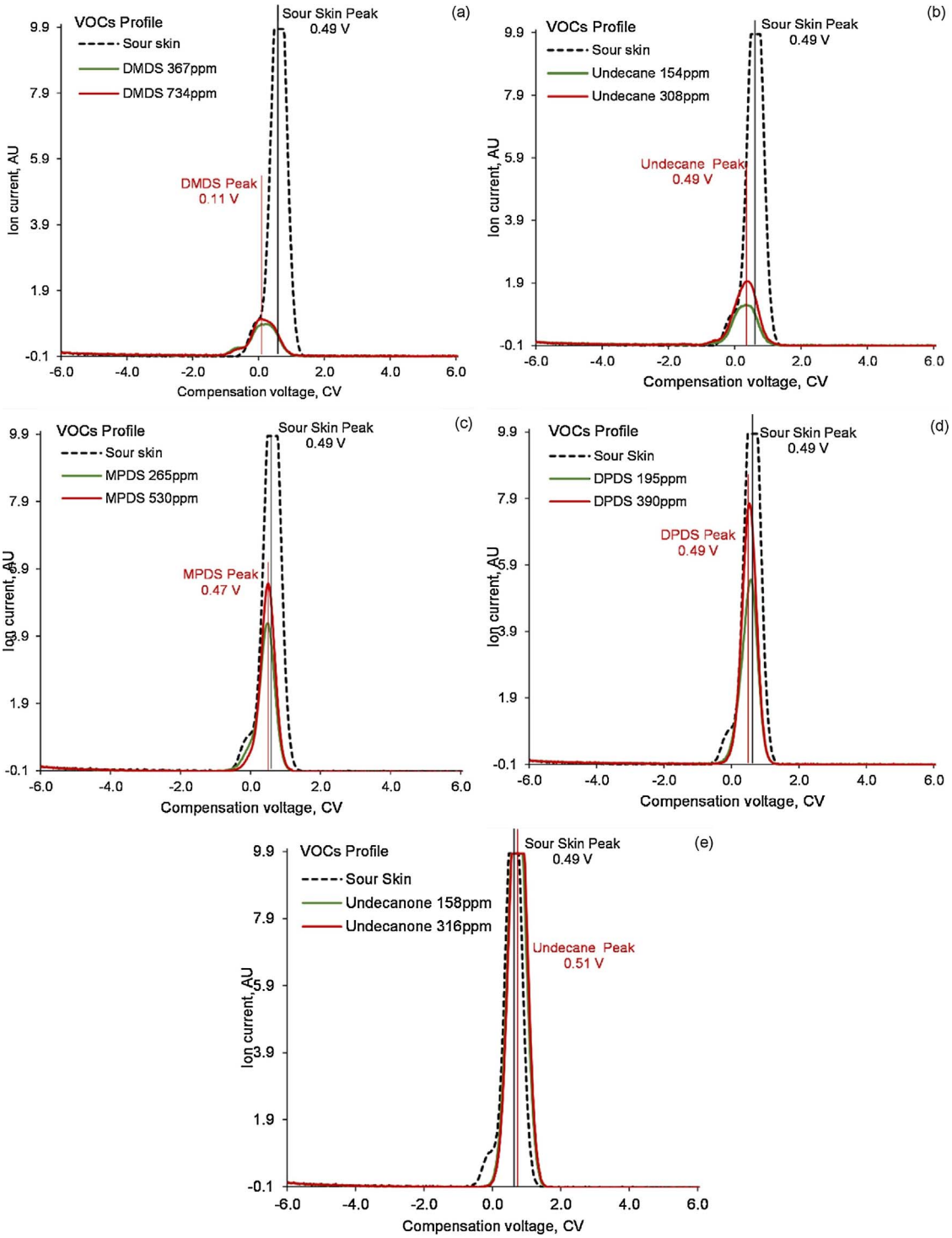

Fig. 6. FAIMS derived ion current plots for typical VOCs standards a) dimethyl disulfide (DMDS), b) undecane, c) methyl propyl disulfide (MPDS), d) dipropyl disulfide (DPDS), and e) 2-undecanone at two different concentrations (the ion current plot of VOCs pertinent to onion sour skin is used as a baseline).

towards right. Wang et al. (2015) studied the effect of humidity on VOCs (ketones, alcohols and aromatics) detection through FAIMS and reported that with the increase in humidity, the major peaks shifted right towards higher CV. The amount of peak shift depends on several factors like humidity of the analyte and quantifying the shift was beyond the scope of the study. Ethyl acetate ion peak, due to high humidity, was also shifted towards right at higher CV (Fig. 5f) compared to potato soft rot pertinent VOCs. The ion peak for ethanol (Fig. 5c) too was found close to the potato soft rot peak indicating its presence in the VOCs released during soft rot progression in potatoes under storage. The ion current plots for 1-butanol (Fig. 5d) and pentane (Fig. 5e) resembled the peak common to both healthy controls and the inoculated treatments, suggesting that pentane and 1-butanol were common to both healthy and inoculated tubers.

The ion current peaks for key VOCs standards associated with progression of *B. cepacia* caused onion sour skin were observed close to the ion current peak for VOCs pertinent to onion sour skin progression under storage. The ion current plots for DMDS, DPDS, MPDS, undecane and 2-undecanone have been reported in Fig. 6. It can be observed that these resembled the only peak in the onion sour skin ion current plot,

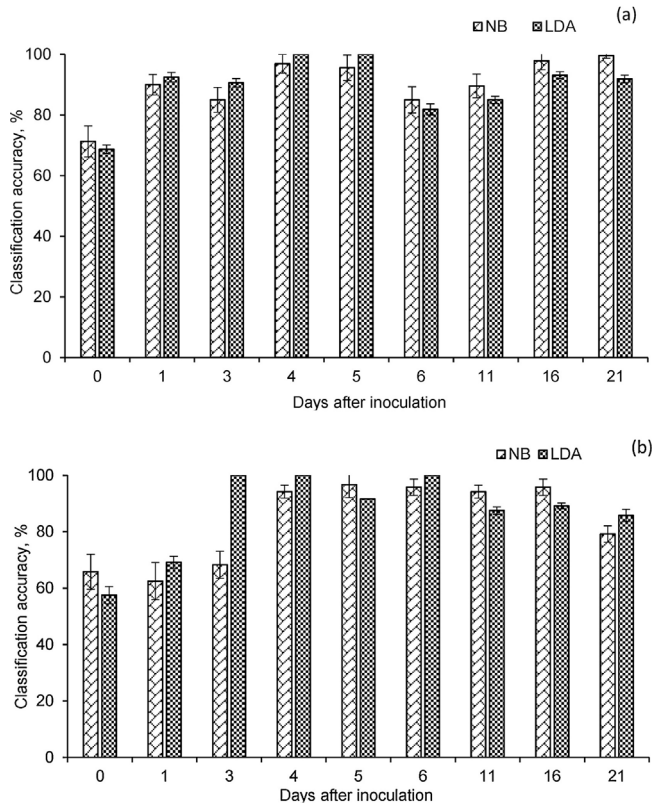

Fig. 7. Average overall classification accuracies for detection of a) soft rot in potatoes caused by *P. carotovorum* and b) sour skin in onions caused by *B. cepacia* using NB (Naïve Bayes) and LDA (linear discriminant analysis) classifiers for different DAIs (days after inoculation).

however, with lower ion current values. It indicated that all these analytes constitute VOCs pertinent to sour skin; however, their concentration in sour skin is greater than what was prepared and tested in this study.

3.4. Classification of FAIMS response

The classification accuracies for both *P. carotovorum* subsp. *carotovorum* caused soft rot in potatoes, and *B. cepacia* caused sour skin in onions using NB and LDA classification models, with 10-fold cross-validation has been reported in Fig. 7. Crop stored under reduced temperature for both potato and onion studies (TP2 and TO2 respectively), were considered as healthy until the infection pertinent VOCs were detected by FAIMS (i.e. 6 DAI for potato soft rot and 11 DAI for onion sour skin). The lowest average overall classification accuracies for detection of *P. carotovorum* subsp. *carotovorum* caused potato soft rot was observed on 0 DAI, when all the treatments were in a similar state of VOCs release. This was also true for average classification accuracies for detection of *B. cepacia* caused onion sour skin. With the progression of storage infection, the FAIMS could pick the biomarker VOCs signatures, which resulted in increased overall classification accuracies in case of both the storage infections. High classification accuracies were reported until 21 DAI. However, for onion sour skin, the classification accuracies for infected treatment reduced on 21 DAI for both the classifiers. This could be attributed to the reduced VOCs emission from the inoculated treatments on 21 DAI compared to 4–16 DAI. Li et al. (2011) also reported that the highest VOCs release was between 3 and 6 DAI in *B. cepacia* caused sour skin in onions stored at 30 °C in an incubator.

4. Conclusions

Applicability of portable FAIMS was tested to detect major infections in potatoes (*P. carotovorum* subsp. *carotovorum* induced soft rot) and onions (*B. cepacia* induced sour skin) and evaluated was the effect of storage conditions. Studies were conducted to evaluate soft rot and sour skin response on a temporal basis for up to 21 day(s) for samples stored at room temperature and reduced temperature of 4 °C. Overall, FAIMS can detect soft rot and sour skin under both storage conditions, when no visible symptoms were apparent on inoculated samples and the olfactory biomarkers release was at trace levels. Temporally, time frame for soft rot detection at room temperature condition was 1 DAI and between 6 and 11 DAI for reduced temperature conditions. For above conditions, the time frame for sour skin detection was 3 DAI and 16 DAI, respectively. In terms of FAIMS training and applicability in bulk storage, PCA based contribution analysis results confirmed a range of significant CV and DF% between −1.3 V to −0.9 V and 52%–72%, respectively for soft rot detection. For sour skin detection, such range was −0.24 V to 0.48 V CV and 44%–77% DF. Classifiers further confirmed the validity of extracted features in above mentioned ranges with overall accuracies greater than 81% after the VOCs pertinent to storage infections were captured by FAIMS. FAIMS response with baseline VOCs confirmed ethanol, acetone, 2-butanone and ethyl acetate possibly contributing to soft rot response whereas pentane and 1-butanol were potentially associated with both healthy and soft rot inoculated tubers. Dimethyl disulfide (DMDS), dipropyl disulfide (DPDS), methyl propyl disulfide (MPDS), undecane and 2-undecanone were found to be associated with healthy controls as well as with sour skin infected onion bulbs.

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References

Alexander, A.A., Pasamontes, A., Peiranao, D.J., Zhao, W., Dandekar, A.M., Fiehn, O., Ehsani, R., Davis, C.E., 2014. Detection of huanglongbing disease using differential mobility spectrometry. Anal. Chem. 86, 2481–2488.

Biondi, E., Blasioli, S., Galeone, A., Spinelli, F., Cellini, A., Lucchese, C., Braschi, I., 2014. Detection of potato brown rot and ring rot by electronic nose: from laboratory to real scale. Talanta 129, 422–430.

de Lacy Costello, B.P.J., Evans, P., Ewen, R.J., Gunson, H.E., Ratcliffe, N.M., Spencer-Phillips, P.T.N., 1999. Identification of volatiles generated by potato tubers (*Solanum tuberosum* CV: Maris Piper) infected by *Erwinia carotovora, Bacillus polymyxa* and *Arthrobacter* sp. Plant Pathol. 48, 345–351.

Eiceman, G.A., Nazarov, E.G., Miller, R.A., Kyrlov, E.V., Zapata, A.M., 2002. Micro–machined planar field asymmetric ion mobility spectrometer as a gas chromatographic detector. Analyst 127, 466–471.

Jansen, R.M.C., Wildt, J., Kappers, I.F., Bouwmeester, H.J., Hofstee, J.W., van Henten, E.J., 2011. Detection of diseased plants by analysis of volatile organic compound emission. Ann. Rev. Phytopathol. 49, 157–174.

Jarvenpaa, E.P., Zhang, Z., Huopalahti, R., King, J.W., 1998. Determination of fresh onion (*Allium cepa* L.) volatiles by solid phase micro–extraction combined with gas chromatography–mass spectrometry. Z. Lebensm. Unters. Forsch. A 207 (1), 39–43.

Jolliffe, I.T., 2002. Principal Component Analysis, Springer Series in Statistics, second ed. Springer–Verlag Inc., New York, NY.

Kjeldsen, F., Christensen, L.P., Edelenbos, M., 2003. Changes in volatile compounds of carrots (*Daucus carota* L.) during refrigerated and frozen storage. J. Agric. Food Chem. 51, 5400–5407.

Konduru, T., Rains, G.C., Li, C., 2015. A customized metal oxide semiconductor–based gas sensor array for onion quality evaluation: system development and characterization. Sensors 15, 1252–1273.

Kushalappa, A.C., Lui, L.H., Chen, C.R., Lee, B., 2002. Volatile fingerprinting (SPME–GC–FID) to detect and discriminate diseases of potato tubers. Plant Dis. 86, 131–137.

Li, C., Schmidt, N.E., Gitaitis, R., 2011. Detection of onion postharvest diseases by analyses of headspace volatiles using a gas sensor array and GC–MS. Food Sci. Technol. 44, 1019–1025.

Naes, T., Isaksson, T., Fearn, T., Davies, T., 2002. A User-friendly Guide to Multivariate Calibration and Classification. NIR Publications, Chichester, U.K.

Ng, A.Y., Jordan, M.I., 2002. On Discriminative Vs. Generative Classifiers: A Comparison of Logistic Regression and Naïve Bayes. http://ai.stanford.edu/%E2%88%BCang/

papers/nips01-discriminativegenerative.pdf/. (Accessed 24 May 2016).
Olsen, N., Miller, J., Nolte, P., 2006. Diagnosis and Management of Potato Storage Diseases. http://www.cals.uidaho.edu/edcomm/pdf/cis/cis1131.pdf/. (Accessed 10 May 2017).
Parris, R., Allsworth, M., Laine, C., 2014. Feasibility Study: Using Owlstone's Lonestar Analyzer for the Detection of Acetic Acid in Crude Oil. http://www.owlstonenanotech.com/sites/default/file/PDF/Acetic%20Acid%20Application%20Note%20(new).pdf/. (Accessed 01 April 2016).
Pelter, G.Q., Sorensen, E.J., 2004. Crop Profile for Onions in Washington. USDA National Information System Pest Management Center.
Potato Council, 2012. Store Managers' Guide. https://potatoes.ahdb.org.uk/sites/default/files/publication_upload/Store%20Managers%20Guide%20Updated%2011.05.12.pdf. (Accessed 16 August 2017).
Prithiviraj, B., Vikram, A., Kushalappa, A.C., Yaylayan, V., 2004. Volatile metabolite profiling for the discrimination of onion bulbs infected by *Erwinia carotovora* ssp. *carotovora*, *Fusarium oxysporum* and *Botrytis allii*. Eur. J. Plant Pathol. 110, 371–377.
Rutolo, M., Covington, J.A., Clarkson, J., Iliescu, D., 2014. Detection of potato storage disease via gas analysis: a pilot study using field asymmetric ion mobility spectrometry. Sensors 14, 15939–15952.
Rutolo, M.F., Iliescub, D., Clarkson, J.P., Covington, J.A., 2016. Early identification of potato storage disease using an array of metal–oxide based gas sensors. Postharvest Biol. Technol. 116, 50–58.
Sankaran, S., Maja, J.M., Buchanon, S., Ehsani, R., 2013. Huanglongbing (citrus greening) detection using visible, near infrared and thermal imaging techniques. Sensors 13, 2117–2123.
Schroeder, B.K., Humann, J.L., du Toit, L.J., 2012. Effects of postharvest onion curing parameters on the development of sour skin and slippery skin in storage. Plant Dis. 96, 1548–1555.
Schwartz, H.F., Mohan, S.K., 2008. Compendium of Onion and Garlic Diseases and Pests, second ed. American Phytopath. Society, St. Paul, MN.
Sinha, R., Khot, L.R., Schroeder, B.K., Si, Y., 2017a. Rapid and non–destructive detection of *Pectobacterium carotovorum* causing soft rot in stored potatoes through volatile biomarkers sensing. Crop Prot. 93, 122–131.
Sinha, R., Khot, L.R., Schroeder, B.K., 2017b. FAIMS based sensing of *Burkholderia cepacia* caused sour skin in onions under bulk storage condition. Food Meas. Charac. http://dx.doi.org/10.1007/s11694-017-9537-y.
Toivonen, P.M.A., 1997. Non-ethylene, non-respiratory volatiles in harvested fruits and vegetables: their occurrence, biological activity and control. Postharvest Biol. Technol. 12, 109–125.
USA Onions, 2016. http://usaonions.com/storage-and-handling/. (Accessed 24 June 2016).
Varns, J.L., Glynn, M.T., 1979. Detection of disease in stored potatoes by volatile monitoring. Am. Potato J. 56, 185–197.
Voss, R.E., Timm, H., 2016. Proper Environment for Potato Storage. http://vric.ucdavis.edu/pdf/POTATOES/potato_storage.pdf/. (Accessed 05 September 2016).
Wang, H., Chen, C., Liu, Y., Zhang, X., Kong, D., Wang, X., Luoc, J., 2015. Humidity effects on resolution and sensitivity of UV–FAIMS in VOCs detection. Anal. Methods 7, 1401–1406.
Waterer, D.R., Pritchard, M.K., 1984. Monitoring of volatiles: a technique for detection of soft rot (*Erwinia carotovora*) in potato tubers. Can. J. Plant Pathol. 6, 165–171.