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Rapid and non–destructive detection of *Pectobacterium carotovorum* causing soft rot in stored potatoes through volatile biomarkers sensing

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ABSTRACT

Early disease detection plays a significant role in the pre– and post–production management of specialty crops, that often are stored for several months prior to consumption. Potato is one of the most important specialty crops of the United States. However, soft rot in potatoes due to pathogenic infections during bulk storage, accounts for substantial losses to the industry. This study was aimed at assessing the applicability of an emerging technology, portable field asymmetric ion mobility spectrometry (FAIMS), towards early detection of soft rot in potatoes during bulk storage. The FAIMS senses mobility of ions pertinent to volatile organic compounds (VOCs) released from inoculated tubers. In this study, potato tubers, inoculated with *Pectobacterium carotovorum* causing soft rot, were analyzed using FAIMS over a 30–day period in storage. Sterile water inoculated tubers were considered as healthy controls. Results suggest that FAIMS can detect soft rot as early as two days after inoculation (DAI) by effectively capturing VOCs associated with rot progression. The activity of pathogen and associated VOCs release was maximum during the second week after inoculation. A principal component analysis showed a clear distinction between the healthy and *P. carotovorum* inoculated tubers. Classification models, quadratic discriminant analysis and Naïve Bayes with leave–one–out cross validation confirmed the validity of FAIMS response with accuracies between 83 and 100% for both healthy and *P. carotovorum* inoculated tubers.

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1. Introduction

Currently, there are approximately 7.4 billion people worldwide, of which around 0.8 billion are undernourished. There has been a decrease in the prevalence of undernourishment from 18.6% in 1990–92 to a projected estimate of 10.8% in 2014–16 (Godfray et al., 2010; FAO Statistical Pocketbook, 2015). Such reduction rate has been attributed to the technological advancements for production agriculture and post–production processes. Postharvest crop loss is one of the most important factors which contributes to global food insecurity. Twenty–four percent of the total crop loss worldwide, and 9% in developed nations occurs during produce handling and storage (Lipinski et al., 2013). Plant diseases are often responsible for major postharvest crop losses (Sciumbato, 1993; Beuve et al., 1999; Thinlay et al., 2000; Flood, 2010). Therefore, effective disease detection and proper postharvest management of crops are key to increasing global food security.

Potato is one of the major staple food crops of the world and the United States (U.S.) is the fifth largest potato producer in the world, with a productivity of about 47.2 metric ton ha^{-1}, reported in 2014 (FAOSTATS, 2015). Around 7.5% of the potatoes produced in the U.S. are lost due to associated storage issues during potato bulk storage. Bacterial soft rot of tubers, caused by members of the soft rot Enterobacteriaceae (SRE), are significant contributors to the storage losses (Olsen et al., 2006). *P. carotovorum* is one major group of bacteria within the SRE that causes soft rot in potatoes. During bulk storage, soft rot is commonly a result of a favorable micro–climatic conditions within the potato pile producing localized “hot spots”. The pathogen can spread quickly to healthy tubers located below such hot spots in the pile, which is facilitated by intense respiration activity and the heat released from rotting tubers (Olsen et al., 2006; Inglis et al., 2011).

During normal growth, most plants produce intrinsic volatile organic compounds (VOCs) released through leaves, flowers and fruits (Tholl et al., 2006). In recent years, the sensing of VOCs for monitoring and early detection of diseases in plants and produce

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has emerged as a promising tool (Cardoza et al., 2002; Toome et al., 2010; Laothawornkitkul et al., 2010; Jansen et al., 2011; Spadafora et al., 2016). Headspace sampling followed by Gas Chromatography–Mass Spectrometry (GC–MS), GC–Flame Ionization Detector (GC–FID), and electronic noses for disease detection in plants and agricultural produce are techniques that have been evaluated and reported by many researchers (Doughty et al., 1996; Arshak et al., 2004; Deng et al., 2004; Ebel et al., 2006; Blasioli et al., 2010; Copolovici and Niinemets, 2010; Konduru et al., 2015).

Analysis of volatiles emitted by *P. carotovorum* inoculated potato tubers can be used for rapid detection of diseases in storage facilities. Varns and Glynn (1979) investigated the feasibility of early disease detection in potato tubers inoculated with *P. carotovorum,* previously known as *Erwinia carotovora*. The GC–MS analysis identified acetone, ethanol and 2–butanone as volatile biomarkers that were produced in large concentrations. Waterer and Pritchard (1984) investigated *Corynebacterium sepedonicum*, now *Clavibacter sepedonicus* (causing ring rot of potatoes) and *E. carotovora* (causing soft rot of potatoes) and reported associated volatile biomarkers to be ethanol, methanol and acetaldehyde. Similarly, de Lacy Costello et al. (1999) studied volatiles associated with *E. carotovora*, *Bacillus polymyxa* and *Arthrobacter* sp., using GC–MS and reported 22 unique volatiles indicative of soft rot caused by *E. carotovora*. Some key volatile biomarkers identified were acetone, 2–propenal, 2–butanone, 1–butanol and 2–pentanone. Kushalappa et al. (2002) investigated volatile release pattern from potatoes (variety: Russet Burbank) inoculated with different subsp. of *E. carotovora* at 3, 4 and 5 days after inoculation (DAI) using the GC–FID technique. They reported varying volatiles' fingerprints based on the retention time (RT) to classify different pathogen groups. Other studies also used different gas analysis techniques to detect potato tuber soft rot under storage conditions (Ouellette et al., 1990; Lyew et al., 1999, 2001; British Potato Council, 2000; Lui et al., 2005). Most of these studies used a headspace sampling method followed by laboratory based GC–MS/GC–FID analysis to characterize the volatiles. Although accurate, the above techniques are not intended for real–time detection, require skilled labor and are often time consuming, costly and do not offer system portability.

Recently, a new emerging technology known as field asymmetric ion mobility spectrometry (FAIMS) is being used in volatile based analysis. FAIMS characterizes volatiles by fingerprinting the ion mobility of constituent ions of volatiles instead of chemically analyzing the VOCs composition. Researchers have studied FAIMS applications in the medical, food and dairy industry (Arasaradnama et al., 2014; Zhao et al., 2015). Alexander et al. (2014) used differential mobility spectrometry (DMS), which is synonymous to FAIMS, to detect huanglongbing (HLB) disease by analyzing the VOCs released from the infected citrus trees. Rutolo et al. (2014) evaluated the feasibility of detection of potato (variety: Maris Piper) soft rot caused by *P. carotovorum* using this technology. However, no study quantified the temporal progression of soft rot in potato tubers under storage conditions. More studies that evaluate portable FAIMS for detecting potato rot in different cultivars, and during varied bulk storage types and environments are needed. Moreover, studies are needed to understand the ability of FAIMS to characterize and quantify the release of volatile biomarkers specific to soft rot on a temporal basis.

In this study, the pattern of release of VOCs by *P. carotovorum* inoculated potato tubers was studied using FAIMS technology. The hypothesis is that the portable FAIMS can detect volatile biomarkers of soft rot before symptoms are visible on the tubers, leading towards early detection and effective disease management. The specific objectives of the study were to assess the applicability of FAIMS towards: 1) detection of potato soft rot caused by *P. carotovorum*, 2) assessing how early the disease symptoms can be detected during storage, and 3) monitoring of the temporal progression of the disease and characterization of associated volatile biomarkers.

2. Materials and methods

2.1. Sample preparation

Russet Burbank is one of the most common potato varieties grown in the Pacific Northwest states of the U.S., with yield ranging from 28 to 67 metric ton ha^{-1} (Potato Association of America (2015)). This variety is considered to be an industry standard and the harvested tubers are often bulk stored for up to 12 months. Therefore, potato tubers (variety: Russet Burbank) from the 2015 growing season and stored in a commercial bulk storage facility (AgriNorthwest Inc., Prescott, WA, U.S.) were used in this study. Tubers were inoculated with *P. carotovorum*. *P. carotovorum* subsp. *carotovorum* strain Ec101 was grown overnight in 5 mL nutrient broth at 28 °C with agitation at 200 rpm. A 0.5 mL aliquot of the culture was then added to 250 mL nutrient broth and incubated overnight at 28 °C with agitation at 200 rpm. Cells were harvested by centrifugation (48,800 × G for 10 min), washed with sterile distilled water, and re–suspended in sterile distilled water to an optical density (OD_{600}) of 0.3 (approximately 1×10^8 CFU mL^{-1}). The cells were then harvested by centrifugation and re–suspended in sterile distilled water at 1/10th the volume (approximately 1×10^9 CFU mL^{-1}) (Vidaver, 1976; Dung et al., 2014). A high concentration of inoculum was used to ensure consistent rot of the tubers. The inoculum was placed in a 15 mL sterile tube and a 22–gauge needle was completely immersed in the inoculum and then used to puncture the surface of sterilized tubers (to a depth of 2.5 cm) five times on the same surface about 1 cm apart. Before inoculation, the tubers were washed with tap water and the injection sites were sterilized by cleaning the surface using a cotton swab with 95% ethanol. A similar protocol was followed to inoculate a set of tubers with sterile water, which were used as healthy controls in the experiment.

2.2. FAIMS set up

2.2.1. The existing set up

Portable FAIMS is an emerging technology for gas analysis. The constituent gases are differentiated based on the different mobility of the constituent ions in a variable electric field. The volatiles to be analyzed are fed into the ionization chamber using carrier gas, which was lab air in this study. These are then ionized using a radioactive source (Ni^{63}) by a charge transfer process and both positive and negative ions are generated. The ion clouds mark their way to the electrode channel where application of a radio frequency (RF) waveform causes the ions to move in a zig–zag trajectory under the varying electrical field. Based on the ion mobility, many ions collide with the electrode plate losing their charge and some ions collide with the detector plate generating an ion current. In addition to the RF waveform, a compensation voltage (CV) (DC Voltage) was also added to modify the RF waveform to shift the trajectories of the ions, so that they reach the detector plate and do not lose their charge through collision with the electrode plates. The value of the CV was increased from −6 V to 6 V in 512 steps, thus generating a spectrum of ion currents corresponding to each CV value. At the same time, the applied RF waveform, known as dispersion field (DF) was varied from 0 to 100% and a 3D spectrum of ion current, CV and DF was generated for both the positive and negative ions in the analyte. Therefore, using FAIMS for detection of desired analytes in a sample comprises three different steps. The

first step is "ionization" in the ionization chamber, followed by "separation" in the electrode channel and finally "detection" by the detector plate (Eiceman et al., 2002; Parris, 2012).

2.2.2. Modifications for volatile sampling

A customized headspace sampling apparatus (Fig. 1) was fabricated to sample the volatiles released from the tubers. A glass jar (capacity 1.0 gallon [3.79 L], Specialty Bottle, WA, U.S.) was used to store replicate samples and was sealed tightly using a stopper ($\phi = 10.16$ cm). Teflon, with high chemical inertness, was used to fabricate the stopper. Teflon tape was used to seal the stopper with the glass jar. Two holes were drilled in the stopper to hold through–the–wall push–to–connect fittings. Teflon tubes were used to connect the sample jar to the FAIMS analyzer (Owlstone Nanotech Ltd., Cambridge, U.K.). One of the tubes was connected to the sample inlet port of the analyzer while the other tube was connected to the clean air outlet. The whole sampling apparatus was held tightly using a custom made fixture to further prevent any leaks from the sampling unit (Fig. 1). The lab air outlet was connected to an air pressure regulator to precisely monitor the pressure of the input air to the scrubber (a filter in the Lonestar analyzer to clean the carrier gas). The circulation of carrier gas and the volatiles in and out of the analyzer is shown in a schematic in Fig. 2.

2.3. Experimental protocol: data collection

In the current study, there were two treatments (sterile water and *P. carotovorum* inoculated tubers), with each treatment having three replicates. Each replicate consisted of 5 tubers placed in the sampling unit (glass jar) and the top of the unit was sealed using food grade cling film, and secured to the neck of the glass jar using a rubber band. The film ensured anaerobic conditions during the sample storage period. All the replicate samples were kept in similar conditions at room temperature (24 °C) in the laboratory. Samples were misted with sterile water before sealing the jar, to maintain a humid environment inside the jar which helped to promote disease progression. A spray bottle (0.7 L volume) was used for this purpose. The sterile water (0.9 mL per trigger × 2) was sprayed on to the tuber samples in the jars. This misting process was repeated on each sampling day, after VOCs evaluation, until day 30.

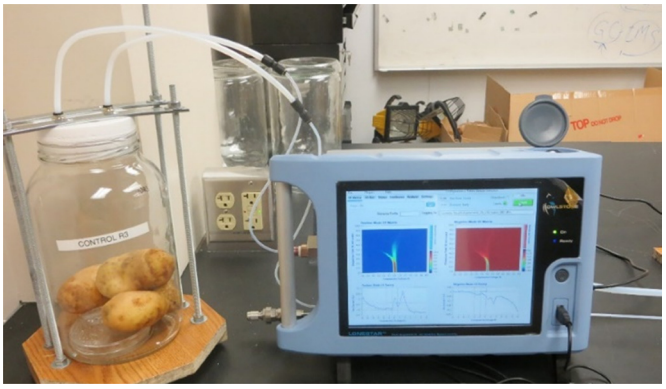

Fig. 1. The experimental setup for the collection and analysis of volatiles using FAIMS.

FAIMS sampling was completed by passing air at a flow rate of 2.0 L min^{-1} and at 0.4 bar pressure of the FAIMS column. The flow of carrier gas is required to drive the ions to the detector plate through the electrode channel, as well as to initialize the process of ionization. The transmission factor (percentage of ions making it to the detector) increases with increasing flow and around 75–80% of ions make it to the detector at an air flow of 1.0–1.2 L min^{-1} (Parris, 2012). Therefore, a higher flow rate (2.0 L min^{-1}) was used to sample the volatiles in order to achieve around 100% transmission factor. Pressure inside the column is required for better ionization inside the ionization chamber. After every sampling, the system was run with air alone to remove all the contaminants from the previous sampling. For cleaning, similar conditions of air flow rate and pressure were followed. For healthy samples, the cleaning time was around 15 min. For the inoculated samples, the cleaning time was less (around 15 min) initially. However, it increased up to 30 min as the soft rot developed within each sampling jar. This was expected, as with temporal disease progression, higher VOCs concentration was released from rotting tubers, requiring a longer period of time to clean out the system.

A total of 6 DF scans per sample were collected on each of the sampling days. The volatiles released from the tubers were initially measured each day for a week and then on days 11, 15, 20, 25 and 30 post inoculation. Monitoring the volatiles each day for first week was in line with the first and second objectives, which were to evaluate the feasibility of the FAIMS to differentiate between healthy and *P. carotovorum* inoculated tubers and to assess the time frame for early detection. The 11 to 30 DAI assessments were towards monitoring the VOCs produced during temporal progression of the soft rot.

2.4. Data analysis

The ion current values in all the obtained scans were initially evaluated using Lonestar software (Owlstone Nanotech Ltd., Cambridge, U.K.). Of the 6 scans per sample per day, 2 of the middle scans, where the ion current value was considered stable, were used for data analysis. Only the positive mode DF matrix was

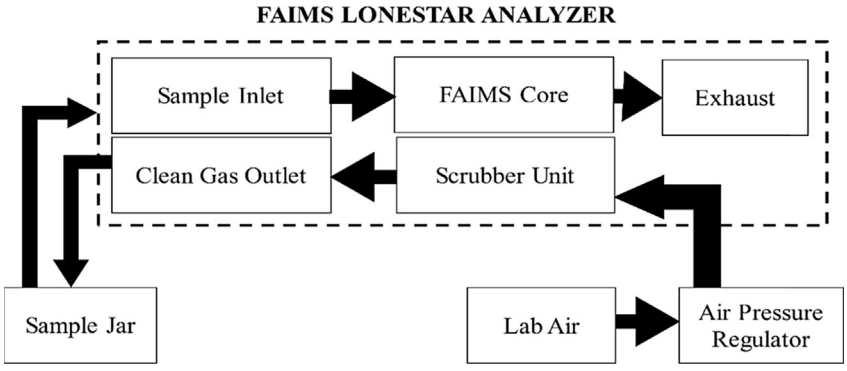

Fig. 2. Block diagram denoting the flow of volatile biomarker ions with carrier gas in FAIMS analysis.

considered in the analysis because no differences between the healthy and inoculated samples were observed in the negative DF matrix. Each scan consisted of a matrix of ion current values corresponding to different DF and CV values. The DF was set to change from 0 to 100% in 51 scanning steps and the CV varied from −6 V to 6 V in 512 steps. Thus, each scan represented a 3D data matrix with 51 × 512 ion current values.

It was observed that most of the differences for water−inoculated and soft rot tubers were in the 30–90% DF and −6.0 V to −0.4 V CV range. For each DF value in the above selected ranges, the maximum ion current had to be extracted for two scans of each sample. An algorithm in MATLAB® (Ver. R2015b, MathWorks Inc., MA, U.S.) was written to extract these value. Raw data were extracted to '.xls' file format using the Lonestar software and was input to the MATLAB® algorithm. The resulting dataset consisted of 144 samples (2 Treatments × 3 Replicates/treatment × 12 days × 2 scans/day) with 31 different DF values, i.e. 144 × 31 size matrix.

A principal component analysis (PCA) was performed to reduce

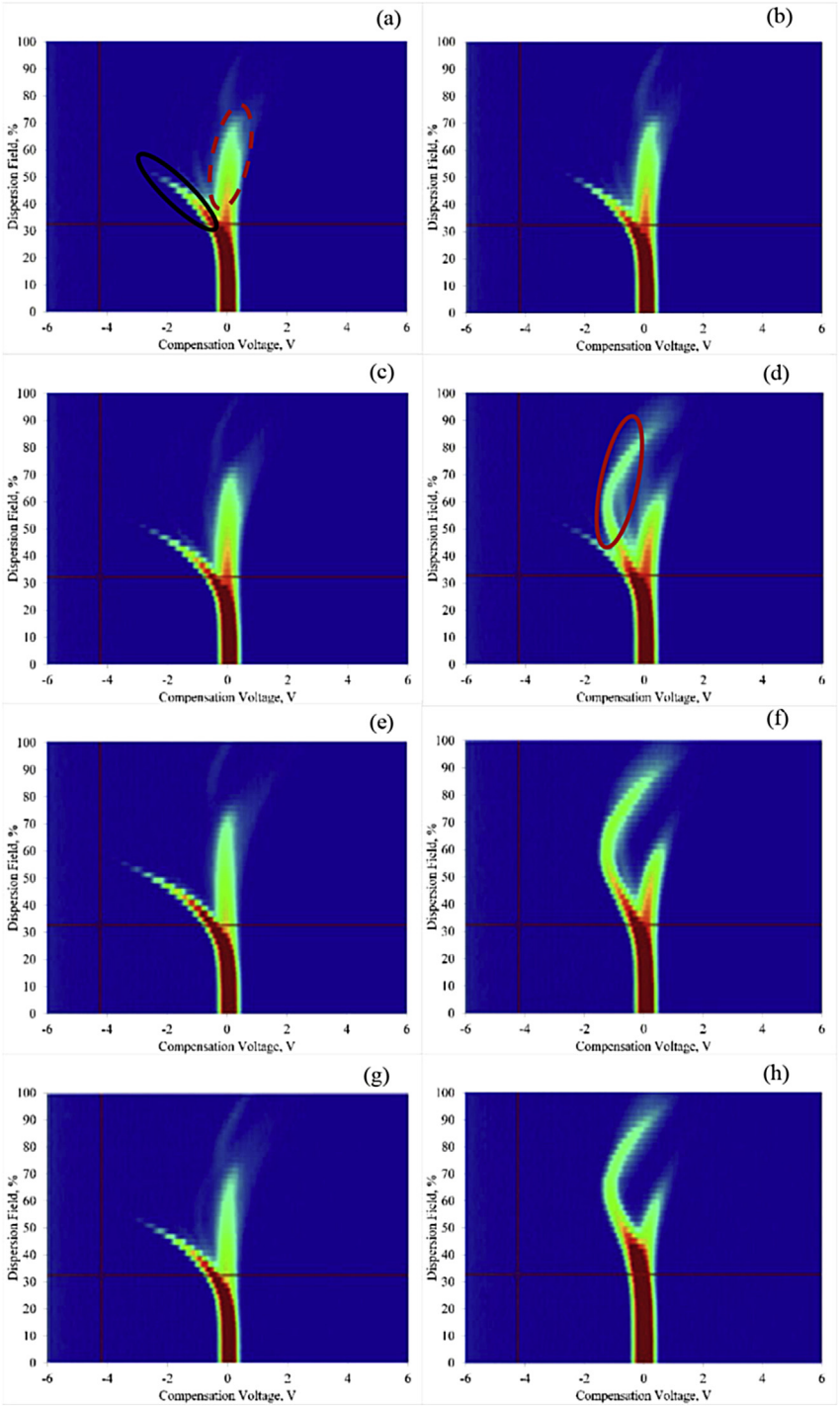

Fig. 3. Ion current plots (Positive DF matrices) for healthy and soft rot infected tubers, respectively, at 0 (a, b), 2 (c, d), 6 (e, f), and 15 (g, h) DAI (Highlighted peaks with elliptical shapes represent RIP [black], VOCs common to both the treatments [red dashed] and soft rot associated VOCs [red]). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

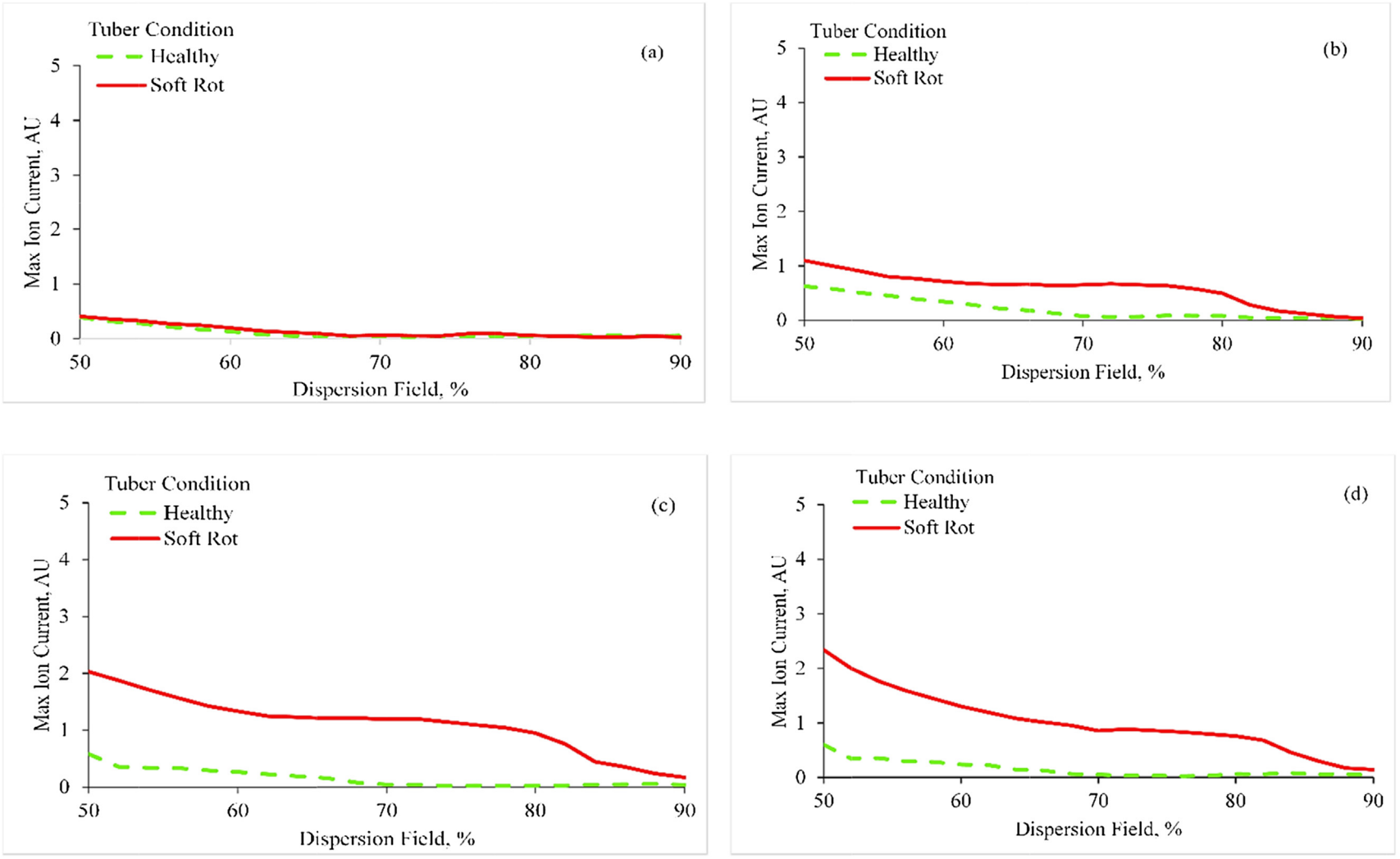

Fig. 4. Comparison of healthy and soft rot infected tubers based on their maximum ion current in positive dispersion field matrices at a) 0, b) 2, c) 6, and d) 15 DAI.

redundancy and understand class discrimination power of the dataset. The PCA works very well in reducing the dimensionality of a dataset consisting of a large number of inter–related variables. However, it retains variation present in the dataset (Jolliffe, 2002; Khot et al., 2011). The variables in the existing dataset were transformed to a new set of variables. This new set of variables consisted of principal components (PCs) of the analysis and were ordered in such a way that they accounted for most of the variation in the non–reduced set of variables.

An algorithm in MATLAB® was written to carry out PCA and to classify the VOCs released from potato tubers. Quadratic Discriminant Analysis (QDA) and Naïve Bayes (NB) classifiers were used to classify the VOCs of healthy and *P. carotovorum* inoculated tubers with leave–one–out (LOO) cross–validation. QDA models the likelihood of each class as a gaussian distribution. NB assigns a probability value to a given class assuming independence among features (Srivastava et al., 2007; Ng and Jordan, 2002). The first three PCs obtained from PCA were used as input features for

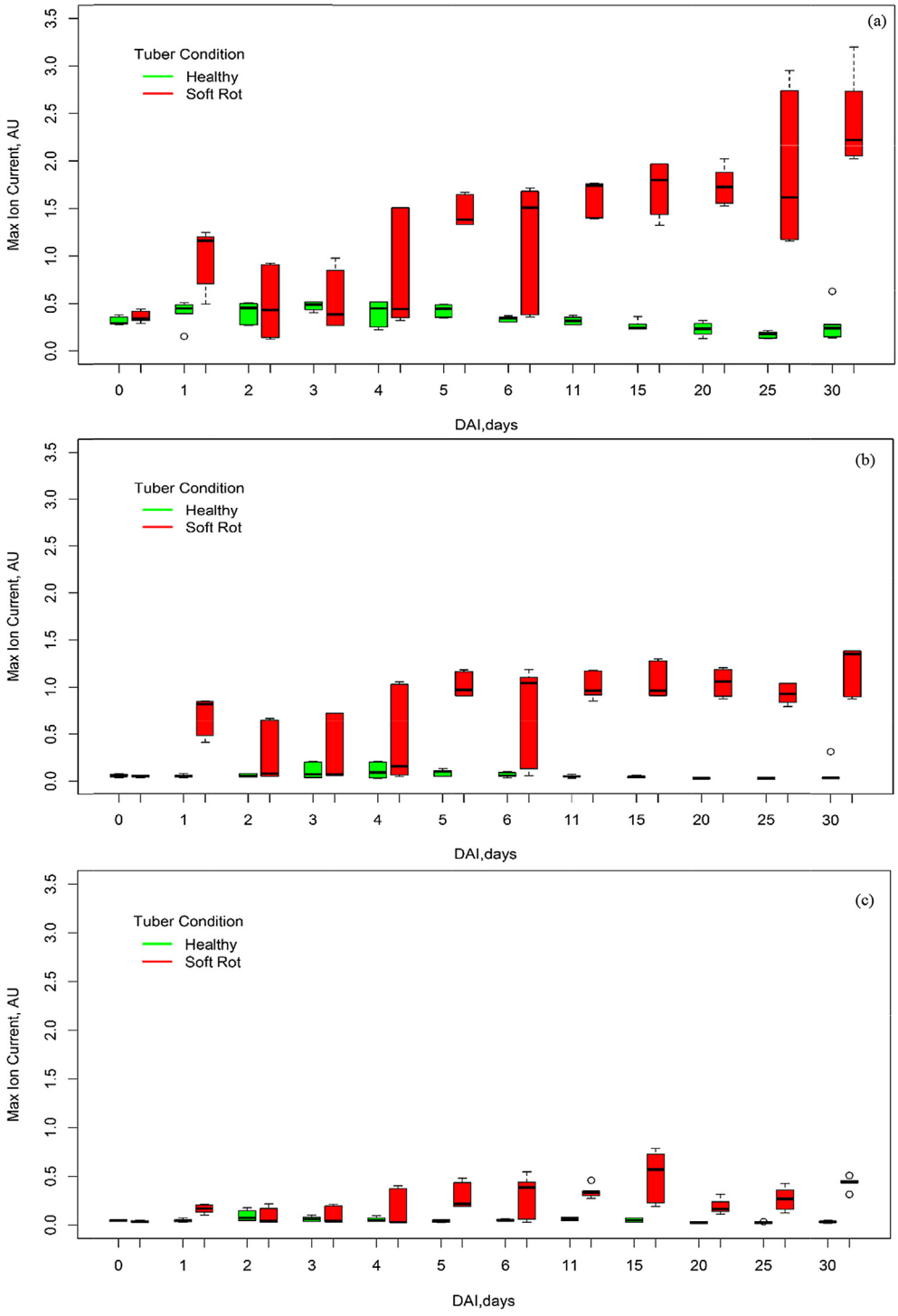

Fig. 5. Box–whisker plots showing the progression of soft rot at a) 54%, b) 70% and c) 84% of the dispersion field.

classification and the results are presented in section 3.2.

3. Results and discussion

3.1. FAIMS derived soft rot responses and temporal monitoring

Fig. 3 shows the ion current plots for the VOCs from healthy and *P. carotovorum* inoculated tubers at different DAI. Each ion current plot (spectrum) represents a 3D matrix giving ion current values corresponding to a particular DF and CV. Although the samples were analyzed until 30 DAI on a temporal basis, only a few representatives of those spectra are shown. On the day of inoculation (0 DAI), the VOCs signatures (Fig. 3a and b) of both the healthy and *P. carotovorum* inoculated tubers are nearly the same indicating that similar VOCs were released by the two groups of tubers. The two highly prominent peaks visible in the spectrum (Fig. 3a) are the peaks corresponding to the reactant ions (highlighted with solid black ellipse in Fig. 3a) and the VOCs common to both healthy and inoculated tubers (highlighted with dashed red ellipse in Fig. 3a). The reactant ion peak (RIP) corresponds to the carrier gas, which is the ambient air.

A third peak in the spectra (Fig. 3d, f, h) of samples from *P. carotovorum* inoculated tubers was visible 2 DAI (highlighted with red ellipse in Fig. 3d). Healthy tubers did not show any such spectral change. It was further observed that the RIP in *P. carotovorum* inoculated tubers was less prominent compared to the healthy control. This may be because the carrier gas is utilized to carry the volatiles to the FAIMS core after ionization and possible clustering of carrier gas ions with the VOCs. With the increase in number of DAI, it was expected that higher quantities of VOCs will be released by the *P. carotovorum* inoculated tubers. This can be visualized by the disappearance of the RIP at 6 DAI and 15 DAI for the *P. carotovorum* inoculated tubers. Overall, results confirmed the suitability of the FAIMS technology to detect soft rot in potato tubers at a very early stage, ranging from 2 DAI to 4 DAI in the different replicates, under the experimental conditions.

The temporal progression of VOCs during the development of soft rot in the *P. carotovorum* inoculated tubers is shown in Fig. 4. The location of an analyte peak on the 3D matrix is a function of both DF intensity and the CV value. It was observed that the analytes present in the VOCs associated with *P. carotovorum* inoculated tubers could be detected at higher DF intensity compared to healthy tubers. Therefore, maximum ion current values corresponding to the 50–90% DF were plotted, and differences between the two treatments were evident (Fig. 3d). As the soft rot pathogen started to act upon the inoculated tubers, the healthy tubers were clearly distinguishable from the *P. carotovorum* inoculated tubers. Such detection was as early as 2 DAI in one of the replicates. This spectrum was characterized by the emission of VOCs pertinent to soft rotting of the inoculated tubers. Separation between the curves corresponding to the heathy and *P. carotovorum* inoculated tubers, respectively, increased with time. This was due to increased VOCs emission from the *P. carotovorum* inoculated tubers. The maximum ion current corresponding to the control samples was found not to change with time.

Fig. 5 presents the comparison of healthy control and *P. carotovorum* inoculated tubers at different DFs namely (a) 54%, (b) 70%, and (c) 84% on a temporal scale of 0–30 DAI. Overall, ion currents corresponding to healthy tubers did not change considerably during 30–day storage at 54, 70 and 84% DF. However, for the *P. carotovorum* inoculated tubers, an increase in the maximum ion current and a high variance in the dataset was observed over time. The variance could be explained by the emission of different quantities of VOCs from the replicates of the inoculated tubers. Thus, FAIMS can be tuned to trigger an alarm if the system detects an increase in ion current at either 54, 74 or 84% DF.

3.2. Class discrimination and classification

In this study, PCA was carried out to assess the sensitivity of FAIMS towards VOCs emitted by healthy and *P. carotovorum* inoculated tubers. The PCA score plot is shown in Fig. 6, where each data

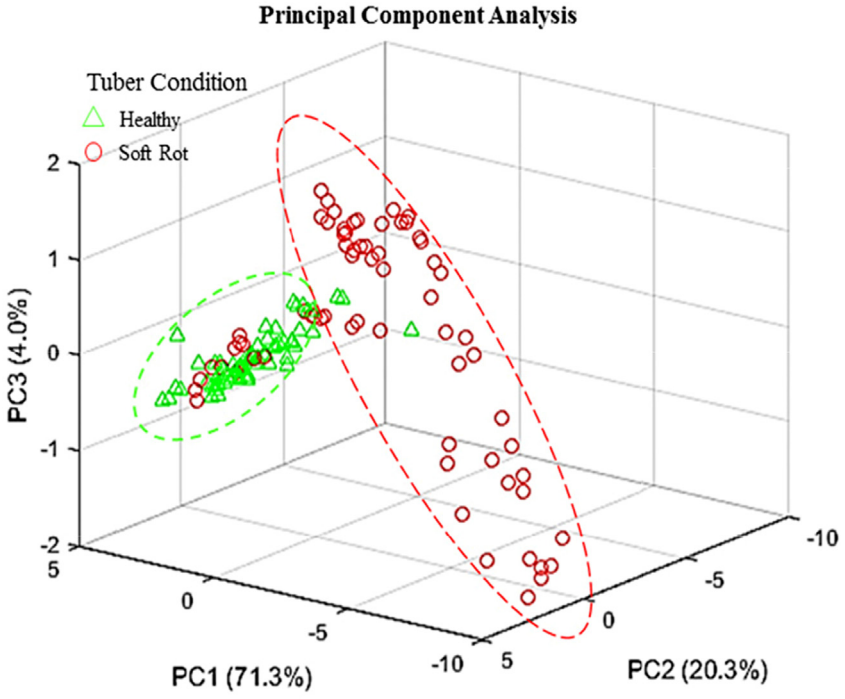

Fig. 6. PCA of the complete dataset showing discrimination ability of the FAIMS captured volatile biomarkers' signatures.

point represents the principal component scores of each replicate sample. The first three PCs accounted for 95.6% of the variability between the healthy and *P. carotovorum* inoculated tubers. Chosen PCs clearly discriminated data on a hyperplane which differentiated the healthy and the inoculated tubers. The maximum ion currents corresponding to the healthy tubers had a maximum spread on the positive side of PC1 and PC2 and on the negative side of PC3. For *P. carotovorum* inoculated tubers, the spread was equal on either side of PC2 and PC3 and mostly towards the negative side of PC1.

A PCA was carried out on each sampling day to assess the separability of FAIMS response ion current values corresponding to healthy and *P. carotovorum* inoculated tubers. Selected score plots, critical to the separability are as shown in Fig. 7. Expectedly, the separability was minimum at 0 DAI as the tubers in both the treatments were identical with a similar state of VOCs release. The separability between the healthy and *P. carotovorum* inoculated tubers increased temporally, with highest separability on 30 DAI. It was also evident from the score plots, that the clusters corresponding to healthy tubers were uncondensed initially (0 and 6 DAI), and grew small and compact as the time progressed (20 and 30 DAI). This could be attributed to the difference in sparsely distributed bacterial colonies on the tubers initially, which were suppressed with unfavorable conditions with time. The clusters for inoculated tubers were not compact which indicated different amounts of VOCs released from different replicates. This could be explained on the basis of different bacterial colony growth in the tubers, which is a common issue in the biological samples.

The pair–wise classification results using QDA and NB classifiers, with LOO cross validation, are presented in Table 1. Overall, the classification accuracies for *P. carotovorum* inoculated tubers were slightly lower initially, which was expected as the rot was under progression in the replicate samples. However, with progression of soft rot, both classifiers reported very high classification accuracies (100%) with QDA performing better than the NB classifier.

Overall results indicate that FAIMS based volatile biomarkers sensing can be a promising technique to detect soft rot caused by *P. carotovorum* in potato tubers under bulk storage conditions. Note

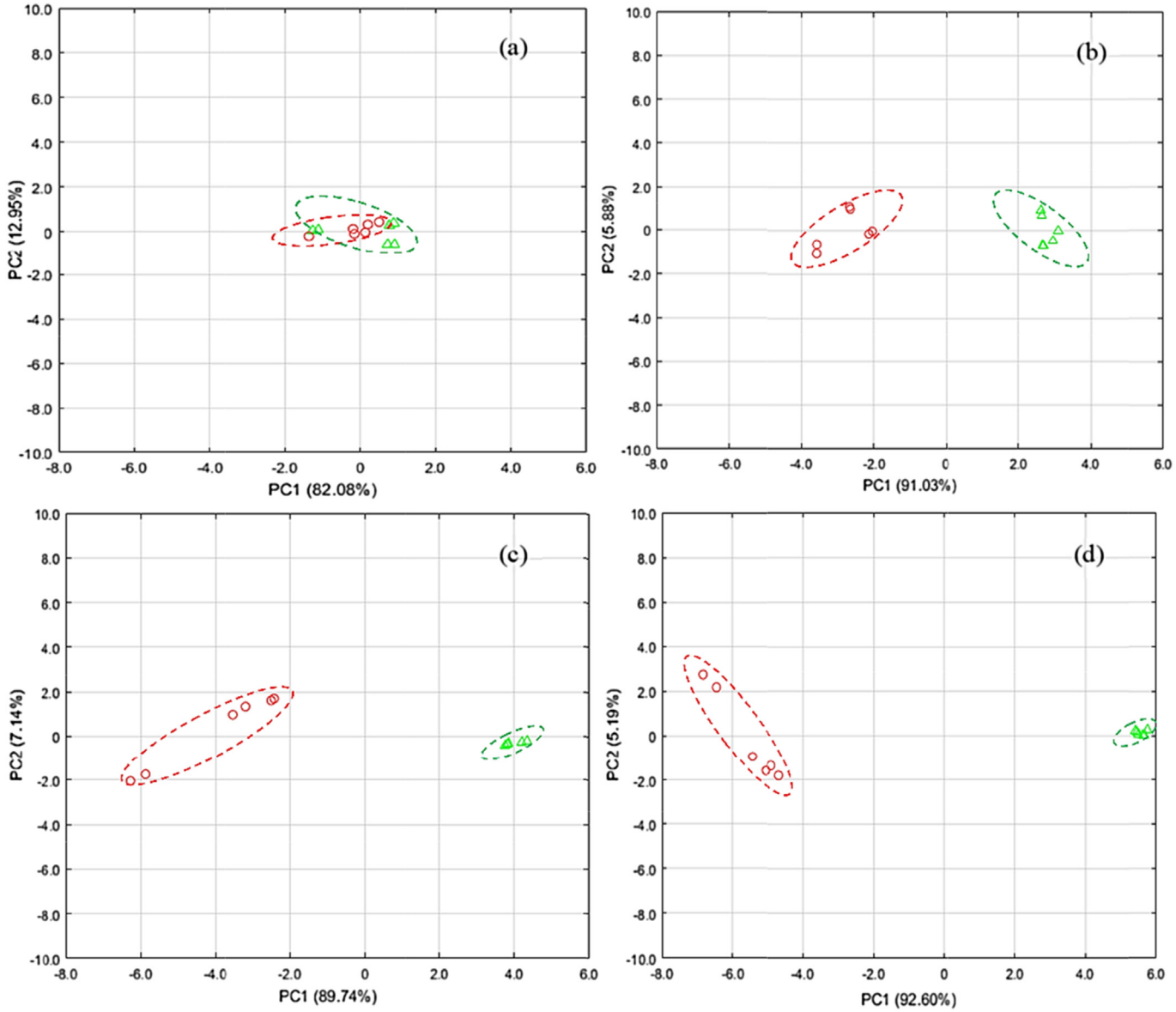

Fig. 7. PCA based class discrimination of healthy (△) and soft rot infected (○) potato tubers at a) 0, b) 6, c) 20, and d) 30 DAI.

Table 1
Classification accuracies (%) for discrimination of healthy and *P. carotovorum* inoculated tubers on different DAI(s).

Classification accuracies (%)						
Time, DAI	Naïve Bayes			QDA		
	Healthy	Soft rot	Overall	Healthy	Soft rot	Overall
1	83.33	83.33	83.33	100.00	83.33	91.67
2	100.00	83.33	91.67	66.67	100.00	83.33
3	100.00	66.67	83.33	100.00	100.00	100.00
4	83.33	83.33	83.33	100.00	100.00	100.00
5	100.00	100.00	100.00	100.00	100.00	100.00
6	100.00	100.00	100.00	100.00	100.00	100.00
11	100.00	100.00	100.00	100.00	100.00	100.00
15	100.00	100.00	100.00	83.33	100.00	91.67
20	100.00	100.00	100.00	100.00	100.00	100.00
25	83.33	100.00	91.67	100.00	100.00	100.00
30	100.00	100.00	100.00	100.00	100.00	100.00

that FAIMS does not undertake chemical analysis of the analytes present in the VOCs and hence, does not quantify what VOCs are being detected. Based on few prior studies that evaluated potato soft rot related VOCs using GC–MS/GC–FID techniques (Varns and Glynn, 1979; Waterer and Pritchard, 1984; de Lacy Costello et al., 1999), the postulation is that acetone, ethanol, methanol, acetaldehyde, 2–butanone, 2–propenal, 1–butanol, 2–pentanone, among others, might be contributing to the FAIMS based ion mobility response. Our ongoing studies are evaluating the response of FAIMS towards the above mentioned standard compounds as well as towards the volatile release variation related at varied bulk storage temperature–humidity conditions during propagation of soft rot caused by *P. carotovorum*.

Real–time detection of soft rot VOCs can be highly instrumental for storage managers/growers in making management decisions for bulk stored produce. Due to the portability offered by FAIMS, it is deemed fit for such a purpose, although the system cleaning time (15 min) can be a deterrent and warrants further modifications to the sampling protocol. Training the FAIMS system to trigger an alarm for real–time detection of soft rot caused by *P. carotovorum* is possible, if the range of DF intensity and CV where ion current values are significantly higher, can be found. The focus of our ongoing studies is to further confirm such ranges. After the alarm for soft rot is raised by FAIMS in the storage facility, storage facility managers can initiate rigorous inspection of the facility. One possible approach might be to use an automated platform integrated with the portable FAIMS system, to continuously sample VOCs inside the facility. This is hypothesized to precisely locate the hot spots of tubers affected by soft rot. Other imaging techniques, such as infrared imaging, might supplement such hot spot mapping of potato piles. Once the infestation regions are located, they can be surgically removed, preventing further crop loss.

4. Conclusions

The feasibility of using FAIMS to detect *P. carotovorum* inoculated tubers was evaluated during a 30–day storage period by monitoring the fingerprints of the VOCs release. It can be concluded that FAIMS was able to detect the soft rot in potato tubers at an early stage, i.e. as early as 2 DAI, when no visible symptoms existed on the tubers and olfactory volatile biomarkers were at trace levels. The VOCs concentration changed temporally, as captured by FAIMS–based maximum ion current data at 54, 70 and 84% DF, and the highest microbial activity was during the second week after inoculation. QDA and NB classifiers confirmed the validity of FAIMS response with classification accuracy for both healthy and *P. carotovorum* inoculated in the range of 83–100%. Our ongoing studies are further evaluating the response of the portable FAIMS to potato soft rot and relating it to the specific volatile biomarkers. Studies are also focused on detailed understanding of volatile release at varied bulk storage temperature–humidity conditions. The focus is to find the significant DF intensity and CV ranges which could be used to train FAIMS for real–time monitoring of soft rot under bulk storage conditions.

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