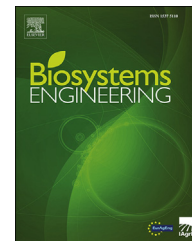


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Research Paper

The use of an electronic nose to detect early signs of soft-rot infection in potatoes

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In this paper we report on the detection of soft-rot in potatoes caused by the bacterium *Pectobacterium carotovorum* through the use of an array of low cost gas sensors. This disease results in significant crop losses in store (circa 5%) with associated negative financial impacts. At present, there is no commercial technological solution for soft rot detection in such stores, with store managers having to regularly inspect large volumes of potatoes. As soft-rot is associated with a strong odour and there is forced air movement through potato stores, our aim was to investigate the potential of an array of low-cost gas sensors to detect the disease. In laboratory conditions, 80 potatoes with and without soft rot (evenly split) were analysed by an array of 11 different gas sensors. These were tested at both pre-symptomatic and symptomatic time points. Results indicated that 100% detection accuracy could be achieved at both time points with only 3 sensors. The identified sensors therefore offer promise for an automated in-store monitoring system.

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1. Introduction

Bacterial soft rot disease caused principally by *Pectobacterium carotovorum* (Czajkowski et al., 2015) results in significant losses in UK potato stores, with approx. 5% of the crop being destroyed each year (AHDB, 2012). At present, there is no technology available for monitoring this disease in commercial stores, but if soft rot could be detected early, the farmer/store manager could make an informed decision of how best to manage the infected crop (usually by selling into the food or animal feed markets, or by changing the storage conditions). Such early identification is not normally possible as potato stores are very large, the tubers are not easily

accessible for visual inspection and the characteristic odour associated with soft rot is only detectable by the store manager when the disease is at an advanced stage. However, we believe that automated detection of soft rot could be achieved through modern gas analysis technology.

This concept is not new, with early work being undertaken by Varns and Glynn (1979) followed by the study of Waterer and Pritchard (1984a,b). These and subsequent studies used either GC (Gas Chromatograph) or GCMS (Gas Chromatograph Mass Spectrometer) in an attempt to identify the specific chemicals that were associated with soft rot (Ratti, Gariépy, & G. R., 1995; Lyew et al., 2001; Kushalappa & Zulfiqar 2001). This resulted in a large number of different potential biomarkers for

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the disease being reported; however due to a range of experimental differences, there is no consensus over their identity. This is not unexpected as it has been reported that plants produce around 200,000 volatiles before and after harvest (Dixon et al., 2002; Fiehn, 2002).

Though these studies are scientifically interesting, they do not provide a solution for practical disease monitoring in potato stores. GC and GCMS are expensive pieces of equipment that require trained staff and significant infrastructure making them unsuitable for a store environment. However, one alternative technology that could be applied is the so called “electronic nose” or “eNose” – an instrument designed to mimic the biological olfactory system. This instrument is already finding favour in precision agriculture, where there is a growing use of sensors and sensor systems to optimise and improve manufacturing in agriculture and forestry (Wilson, 2013). The eNose is relatively cost effective as it can be formed from an array of low-cost chemicals sensors (sub \$50), it uses air as carrier gas, can be produced to be portable (even battery powered) and can provide a simple and quick answer to a chemical identification task. This is in stark contrast to higher-end analytical techniques, such as GC-MS. The number of agricultural applications for eNose that have been studied is considerable, from crop protection, floral odours, ecosystem management to wood management and beyond (Wilson, 2013). In relation to potato soft-rot detection, there have only been a small number of researchers using eNoses (Biondi et al., 2014; De Lacy Costello et al., 2001; Sinha, Khot, Schroeder, & Si, 2017). We have previously demonstrated that early signs of soft rot infection could be detected using ion mobility spectrometry (specifically using an Owlstone Lonestar, UK) and a commercial electronic nose (AlphaMOS Fox 3000, France; Rutolo, Covington, Clarkson, & Iliescu, 2014, 2016). Both of these studies have shown the potential of gas analysis, but have practical issues. The former, though sensitive, uses a technology that is well beyond the financial reach of the potato industry and also requires the use of clean air and a clean environment to operate. The work with the AlphaMOS system showed that it is possible to achieve similar results with an array of gas sensors. However, this system is no longer available (production stopped in 2016) and the exact manufacturers of the sensors are unknown. In addition, these units are constructed from an array of power-hungry, thick-film metal-oxide gas sensors. This severely limits their use in portable/battery powered applications.

Therefore the challenge of developing a dedicated eNose system that can be deployed, within a storage setting for the detection of soft-rot, still remains. To achieve this, it is important to understand how and which low-cost gas sensors respond to the disease and if they will map onto store environments. Furthermore, as most gas sensors are designed to detect inorganic gases (unlike previous work which focussed on organic compounds), new insights may be gained relating to the biomarkers released by the soft rotting bacterium itself or products associated with the enzymatic breakdown of the potato tissue (Smadja et al., 2004). Thus, the main aims of this paper were to identify low cost gas sensors that can detect soft-rot disease and which inorganic gases may play an important role as biomarkers for infection.

2. Materials and methods

2.1. Electronic nose system

The majority of electronic nose instruments, in either a commercial or research setting, deploy an array of metal-oxide gas sensors, numbering 6 to 32. The reason for this is that metal-oxide sensors historically have had a higher sensitivity to a target gas than other sensors. However, the latest generation of electrochemical gas sensors are now achieving similar sensitivities, whilst offering many of the advantages of such sensors. Electrochemical gas sensors have found favour within the industrial safety market and more recently in both indoor and outdoor air quality applications (Mead et al., 2013). Their key advantages include being relatively low-cost (under \$50 per sensor), ultra-low power consumption (they generate energy as part of the detection process), room temperature operation and good tolerance to environmental changes (specifically changes in temperature and humidity).

Furthermore, in this specific application, they map extremely well onto a low temperature potato store environment. Temperatures as low as 0 °C result in a reduction in sensor zero current (the output of the sensor when not being presented with a target gas) and results in a lower limit of detection. In addition, these sensors are tolerant to both wide ranges of humidity and to high humidity due to the way they are constructed.

In this study, we used an in-house electronic nose called the WOLF 4.1 (Warwick OLFaction, with the number referring to the instrument being desktop). The nine sensors selected for testing (Table 1) were all from a special group that are commercially available and specifically designed for outdoor air quality monitoring and thus have very high sensitivity. This array was augmented with additional gas sensors to evaluate if other potential low-molecular weight biomarkers could be identified, specifically carbon dioxide and methane/hydrocarbons, which cannot be easily detected using electrochemical means. The sensors were mounted inside a large case, which included fluidic components, valves (ETO-12, Clippard, USA) and flow sensors (Honeywell AWM-3300) and a single PC board. The sensors used commercial interface boards (either an ISB or Digital Transmitter Board, AlphaSense, UK) that produce either a voltage or current output. Any currents are converted to an output voltage and then the output of all the sensors was measured by a National Instrument DAQ card (USB-6009). The unit is controlled by a custom written LabVIEW program (version 2015, National Instrument, USA) that allows the sensor data to be stored to a file for later analysis.

2.2. Sample preparation

The potato variety chosen for all experimental work was ‘Maris Piper’, due to its widespread use in the industry. The *P. carotovorum* isolate (SBEU_08) used was originally isolated by Dr Glyn Harper (AHDB Potatoes, Sutton Bridge Crop Storage Research) from an infected potato tuber (variety Marfona) showing characteristic symptoms of bacterial soft rot. In pure culture, it caused pitting in Crystal Violet Pectate agar at 27 °C and identity was further confirmed as *P. carotovorum* by PCR

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