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Assessing the antimicrobial potential of aerosolised electrochemically activated solutions (ECAS) for reducing the microbial bio-burden on fresh food produce held under cooled or cold storage conditions

R.M.S. Thorn [a], J. Pendred [b], D.M. Reynolds [a, *]

[a] Centre for Research in Biosciences, Department of Applied Sciences, University of the West of England, Bristol, Frenchay Campus, Coldharbour Lane, BS16 1QY, UK
[b] Pendred Humidification and Water Systems, Worsley Bridge Rd, London, SE26 5BN, UK

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ABSTRACT

The main aim of this study was to assess the antimicrobial efficacy of electrochemically activated fog (ECAF) for reducing the microbial bio-burden on artificially inoculated fresh produce held under cooled (cucumber and vine tomatoes) or cold (rocket and broccoli) storage conditions. The ECAF treatment (1100 ± 5 mV ORP; 50 ± 5 mg L^{-1} free chlorine; 2.7 ± 0.1 pH) resulted in a significant log reduction in the potential pathogen *E. coli* recovered from rocket (2.644 Log_{10} CFU g^{-1}), broccoli (4.204 Log_{10} CFU g^{-1}), cucumber (3.951 Log_{10} CFU g^{-1}) and tomatoes (2.535 Log10 CFU g-1) after 5 days. ECAF treatment also resulted in a significant log reduction in potential spoilage organisms, whereby a 3.533 Log_{10} CFU g^{-1}, 2.174 Log_{10} CFU g^{-1} and 1.430 Log_{10} CFU g^{-1} reduction in presumptive Pseudomonads was observed for rocket, broccoli and cucumber respectively, and a 3.527 Log_{10} CFU g^{-1} reduction in presumptive *Penicillium* spp. was observed for tomatoes (after 5 days). No adverse visual effects on produce were recorded. The results of this study will inform industrial scale-up trials within commercial facilities (assessing shelf-life, microbial quality and organoleptic assessment) to assess the developed ECAF technology platform within a real food processing environment.

1. Introduction

In post-harvest fresh produce processing and manufacturing, effective microbiological management is essential for the control of spoilage organisms, environmental pathogens and foodborne diseases (Hammond et al., 2015). To maintain produce quality and reduce waste, intervention at all stages of the supply chain is important and must involve a multifaceted, integrated approach. Consumption of fresh food produce has increased substantially over the last few decades, but this has been linked with an associated increase in foodborne disease outbreaks (Olaimat and Holley, 2012). This contamination event can occur at any point during the food chain, whereby potentially pathogenic organisms can persist for long periods, both within soil environments and on the fresh food produce itself (Olaimat and Holley, 2012). This is likely the result of biofilm formation (Olmez and Temur, 2010; Niemira and Cooke, 2010), and is of serious concern, given that biofilm structures are known to be more resistant to post-harvest treatments (Aruscavage et al., 2006), including the chlorine washes which are widely used within the food processing industry (Houdt and Michiels, 2010).

The use of antimicrobials is essential in the control of food spoilage organisms and potentially pathogenic foodborne organisms (Holah et al., 2002), and the importance of antimicrobial inclusion in post-harvest washing solutions has been demonstrated using quantitative modelling (Danyluk and Schaffner, 2011). Post-harvest produce is often washed in chlorine to reduce the microbial bioburden, both to improve shelf-life (Meireles et al., 2016) and to target and inactivate potential pathogens (Warriner et al., 2009). Industry typically utilises chlorine solutions of sodium hypochlorite (pH 6.5) applied at concentrations between 50 and 200 ppm free chlorine, for a contact time of between 1 and 5 min (Goodburn and Wallace, 2013). However, high levels of organic loading have been shown to reduce the efficacy of 30–50 ppm chlorine when used as

Abbreviations: ECAS, electrochemically activated solution(s); ECAF, electrochemically activated fog.

* Corresponding author.
E-mail address: Darren.Reynolds@uwe.ac.uk (D.M. Reynolds).

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a produce washing solution (Zhang et al., 2009), and the presence of organic loading itself can lead to the production of harmful by-products (Bull et al., 2011), including trihalomethanes and haloacetic acids (Shen et al., 2016). Hence, alternative chemical approaches (e.g. quaternary ammonium compounds, ozone and hydrogen peroxide), biological methods (e.g. bacteriophage, bacteriocins and enzymes), physical-interventions (e.g. UV-light, temperature and ionising radiation) or combinatorial approaches (Meireles et al., 2016; Warriner et al., 2009; Olmez and Kretzschmar, 2009; Goodburn and Wallace, 2013) are now being developed.

One emerging technology within the food industry are electrochemically activated solution(s) (ECAS; variously named electrolysed oxidising water or electrolysed water). ECAS are generated through the electrolysis of a low salt solution within an electrochemical cell which can be configured to produce solutions with a variety of physicochemical properties (Thorn et al., 2012). These solutions have been shown to be extremely fast acting (Robinson et al., 2011) with broad spectrum antimicrobial activity (including bacterial spores; Robinson et al., 2010). Additional benefits of the use of ECAS include in situ generation from inexpensive raw materials coupled with environmental compatibility (Thorn et al., 2012). Numerous studies have demonstrated the potential use of ECAS within the fresh food produce industry for controlling foodborne pathogens on onions (Park et al., 2008), lettuce (Park et al., 2001; Abadias et al., 2008; Guentzel et al., 2008; Keskinen et al., 2009), tomatoes (Bari et al., 2003; Deza et al., 2003), pears (Al-Haq et al., 2002), peaches (Al-Haq et al., 2001), apples (Okull and Laborde, 2004), strawberries and cucumbers (Koseki et al., 2004a).

Effective antimicrobial action is impacted by concentration, contact time, contact surface and organic loading (Russell, 2004). Aerosol delivery technologies, whereby solid particles or liquid droplets are suspended in a gas, have been shown to be an effective delivery mechanism for a range of antimicrobials, including ECAS (Thorn et al., 2013). Within the food industry application of antimicrobials is mainly via liquid or spray delivery systems; however, over wetting of produce can result in deterioration of the produce and potentially expedient the spoilage process. The effectiveness of aerosol delivery of hydrogen peroxide, sodium hypochlorite, citric acid or ethanol for reducing postharvest diseases has been shown in strawberries (Vardar et al., 2012). This biocidal delivery mechanism has also been successfully utilised to reduce the microbial bioburden of figs (Karabulut et al., 2009), and the presence of *Penicillium digitatum* within a citrus degreening room (Smilanick et al., 2014). However, aerosol delivery of ECAS has not been previously investigated within the food industry.

The main aim of this study was to assess the antimicrobial potential of aerosolised ECAS for reducing the microbial bio-burden on fresh produce held under cooled (cucumber and tomatoes) or cold (rocket and broccoli) storage conditions for up to 5 days. This technology platform is currently being developed as part of an integrated microbial management system to improve shelf-life by controlling spoilage and pathogenic organisms within post-harvest produce. Ultimately, such approaches will contribute to food security and food safety.

2. Materials and methods

2.1. Growth and maintenance of target micro-organisms

Pseudomonas syringae (*Pseudomonas syringae* pv. Phaseolicola), *Escherichia coli* (ATCC 10536) and *Pencillium expansum* (IMB 11203/DSM 62841) were stored at −80 °C until required. *P. syringae* was recovered onto King's B medium (Sigma-Aldrich Ltd., Dorset, UK) at 25 °C, *Escherichia coli* was recovered onto nutrient agar (CM0003; Oxoid, Basingstoke, UK) at 37 °C and *P. expansum* was recovered on potato dextrose agar (CM0139; Oxoid, Basingstoke, UK) at 25 °C. *P. expansum* spores were prepared by incubating spread plate cultures for 10 days at 25 °C. Plate cultures were scraped into sterile distilled water, filtered through glass wool (Sigma-Aldrich Ltd., Dorset, UK) and the resultant suspension washed three times by centrifugation. Fungal spore density was determined by haemocytometry (C-Chip; Incyto, Cheonan, Korea).

2.2. Fresh food produce

All tomato and cucumber fresh food produce samples were supplied by Thanet Earth (Kent, UK). All broccoli fresh food produce samples were supplied by Manor Fresh (Lincolnshire, UK). All rocket samples were supplied by Laurence J Betts (Kent, UK). Sub samples of batches of fresh produce was assessed microbiologically before use within each experimental trial. Each test sample was prepared to a standardised weight of 25 ± 1 g, involving the weighing of an appropriate quantity of rocket and tomatoes and the sterile sectioning of cucumber and broccoli samples prior to inoculation.

2.3. Experimental system for delivery of ElectroChemically Activated Fog (ECAF)

The experimental system is shown Fig. 1. Acidic electrochemically activated solution(s) (ECAS[a]) were generated by the electrolysis of 1% (w/v) NaCl solution within a commercial ECAS generator (Bridge Biotechnology Ltd., Fife, UK). The redox potential and pH of ECAS[a] was measured using redox and pH probes (Sentek, Braintree, UK) connected to a dual channel benchtop meter (Orion™ Dual Star™, Thermofisher, Loughborough, UK). The free chlorine level of generated ECAS[a] was determined using the DPD test (Palintest Ltd., Gateshead, UK). The redox potential of ECAS[a] during production was standardised to 1100 mV (±5 mV). The ECAS solution was aerosolised using an ultrasonic piezoelectric transducer based fogging technology platform (HU-25OG; Pendred Humidification and Water Systems, London, UK) delivering a droplet size of 1–5 μm. The piezoelectric transducer system output was connected to a temperature controlled incubator with integrated racking (MIR-253; Sanyo, Osaka, Japan) to hold fresh food produce. This experimental system was capable of switching between aerosolised Reverse Osmosis (RO) water (Pendred Humidification and Water Systems, London, UK) and electrochemically activated fog (ECAF) treatment regimens at the desired daily time points via a hygrostat control system (DZR-45; Pendred Humidification and Water Systems, London, UK).

2.4. Aerosolisation test procedure

Prior to treatment exposure, produce was inoculated with an artificial microbial load to simulate contamination (Olaimat and Holley, 2012). Rocket, broccoli and cucumbers samples were inoculated with 5.0 Log_{10} CFU g^{-1} of *E. coli* and 5.0 Log_{10} CFU g^{-1} of *P. syringae*. Tomato samples were inoculated with 5.0 Log_{10} CFU g^{-1} of *E. coli* and 4.0 Log_{10} spores g^{-1} *P. expansum*. Inoculated produce was transferred to the experimental test chamber (day 0), and subjected to one of the following treatment regimens: no treatment (control); aerosolised RO water (control) for 24 h a day for 5 days; or ECAF for 8 h (followed by 16 h of aerosolised water) a day for 5 days (test). Experimental regimen and produce types were tested sequentially and the chamber was disinfected between experiments. Rocket and broccoli samples were incubated at 4 °C (cold storage), cucumber and tomato samples were incubated at 14 °C (cooled storage), to replicate industry practice. During