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.

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Abstract *(not more than 200 words)*

The main aim of this study was to assess the antimicrobial potential 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. In addition, ECAF treatment 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.

Keywords

Electrochemically activated solutions; fogging; disinfection; fresh produce

Highlights

Guidance: Highlights are mandatory for this journal. They consist of a short collection of bullet points that convey the core findings of the article. Please include 3 to 5 bullet points (maximum 85 characters, including spaces, per bullet point).

- Effect of electrochemically activated fogs (ECAF) on fresh produce was investigated
- An ECAF delivery technology platform was developed
- ECAF was shown to significantly reduce the microbial bio-burden on fresh produce
- Study results will inform industrial scale-up trials within commercial facilities

1. Introduction[1]

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 50-200 ppm free chlorine, for

[1] **Abbreviations used within this article:**
ECAS = electrochemically activated solution(s)
ECAF = electrochemically activated fog

a contact time of between 1-5 minutes (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 a produce washing solution (Zhang *et al.*, 2009), and the presence of organic loading itself can lead to the production of harmful bi-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 as a mean of controlling food spoilage, storage-life and shelf life of post-harvest produce, whilst also minimizing microbiological contamination from contact surfaces to reduce waste and improve shelf-life, 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 figure 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 a 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; Pendered 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 (Pendered 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; Pendered 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 hours a day for 5 days; or ECAF for 8 hours (followed by 16 hours 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 experimentation, fresh produce samples (25 g samples; n=3) were

removed on day 0, day 1, day 2, day 3, day 4 and day 5. Produce samples were immediately transferred to separate stomacher bags containing 225 mL Letheen broth containing 5 g L^{-1} sodium thiosulphate (neutraliser), stomached for 30 seconds (to break down sample material) and incubated for 20 minutes to neutralise any remaining biocide. Viable counts were performed on selective and differential 1 mL pour plates of neat and diluted recovery suspensions. All produce samples were plated onto Plate Count Agar (CM325B; Oxoid, Basingstoke, UK), used for recovery of aerobic mesophilic microorganisms (all produce). Brilliance *E. coli*/ Coliform Selective Agar (CM1046; Oxoid, Basingstoke, UK) was used for presumptive selective and differential recovery of *E. coli* (all produce). Rocket, broccoli and cucumber samples were plated onto Pseudomonas agar base (CM0559; Oxoid, Basingstoke, UK) with added CFC selective agar supplement (SR0103; Oxoid, Basingstoke, UK), for selective recovery of Pseudomonads. Tomato samples were plated onto Dichloran Rose Bengal Chloramphenicol agar (CM0727; Oxoid, Basingstoke, UK), used for presumptive selective and differential recovery of *Penicillium* sp. via morphological identification. All results were expressed as cfu per g of produce and the minimum detection limit for the assay was 1.0 $\log_{10}$ CFU g^{-1}.

2.5 Analysis of results

To determine whether aerosolisation treatment resulted in significant reductions in viable bacterial cells, an Analysis of Variance (ANOVA) was performed on microbial recovery counts followed by a Dunnett's Multiple Comparison Test against the controls, and a Tukey's test to compare different treatments, with a $p<0.05$ regarded as significant. Graph construction, and statistical analyses were conducted with the use of GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, CA, USA).

3. Results and Discussion

An integral part of this research was the development of a piezoelectric transducer based fogging technology platform, such that any ECAF treatment regimen could be configured and left to operate autonomously (shown in figure 1). This enabled automated delivery of electrochemically activated fog (ECAF) or RO water fog for pre-defined treatment durations. The piezoelectric transducer (resonating frequency ~1.6MHz) utilises ultrasonic waves that are focused on ECAS, generating a relatively dry fog (ECAF) with a droplet size of 1-5 µm volume medium diameter. It was important to standardise this technology platform to ensure delivery of a fog that resulted in no more than micro-condensation on the surface of produce to prevent over-wetting, in accordance with standard industrial practices. ECAF was delivered over an 8 hour period during the night, to simulate what is achievable within an industrial setting.

Based on our experience of using ECAF to decontaminate material surfaces (Thorn *et al.*, 2013) and initial experimentation as part of this study, an 1100 mV ECAS[a] was chosen for aerosolisation. ECAS[a] generated at higher ORP values (>1130 mV) was shown to cause visual discolouration of some produce, particularly leafy produce (e.g. rocket), akin to that previously observed when acidic electrolysed water ice with high concentrations of chlorine has been used to store lettuce (Koseki *et al.*, 2004b). No adverse visual effects were observed on the quality of the fresh produce as result of storage in 1100 mV ECAF within this study, although future studies will include full organoleptic assessment of produce held within ECAF environments. The ORP, pH and free chlorine of generated ECAS[a] was monitored on a daily basis during experimentation by taking samples from the ECAS storage tank (see figure 1), whereby the physicochemical properties

were found to be 1100 ± 5 mV ORP, 2.7 ± 0.1 pH, 50 ± 5 mg L^{-1} free chlorine. At this pH, the dominant free form of chlorine within ECAS[a] will be hypochlorous acid (HClO) and Cl_2, whereby previous studies investigating electrolysed water ice have found that chlorine gas release is important in maintaining bactericidal activity (Koseki *et al.*, 2004b; Wang *et al.*, 2014). Within this study it is not known whether the aerosolisation processes results in the Cl_2 being driven off or is a contributing factor to the antimicrobial potential of ECAF. However, it is almost certainly a combination of active moieties (including HClO and hydroxyl radicals) that will contribute to any resultant antimicrobial activity at the surface of produce tested (Thorn et al., 2012).

Initial microbial assessment of sub-samples fresh food produce batches found there to an average total viable microbial count of 7.02 ± 0.23 Log_{10} CFU g^{-1} for rocket, 5.27 ± 0.17 Log_{10} CFU g^{-1} for broccoli, 4.32 ± 0.07 Log_{10} CFU g^{-1} for cucumber and 0.51 ± 0.77 Log_{10} CFU g^{-1} for tomatoes. Although the background microbial flora loading was produce dependent, the developed inoculation protocol significantly increased the microbial bioburden of specific pathogenic and spoilage organisms present on all fresh food produce samples tested.

Figure 2 shows the microbial recovery results on each sample day (0-5) for rocket post-exposure to no treatment, RO water fog treatment or 1100 mV ECAF (test) treatment under cold storage conditions (4°C). There was no significant difference between no treatment and RO water fog treatment regimens for any sample day for the total viable microbial count (TVCs) recovered from rocket samples (figure 2a). In contrast, there was a significant log reduction on all sample days when comparing rocket held in ECAF cold storage compared to both no treatment and RO water fog treatment controls, whereby the log reduction increased with increasing ECAF treatment exposure (i.e. as a factor of time; table 1). Surprisingly, there was a significant log reduction in recovered presumptive *E. coli* from rocket stored in the RO water fog compared to those stored in

the no treatment control on day 2, day 3 and day 4 (figure 2b). Since RO water it not known to be antimicrobial against *E. coli*, it is postulated that this is purely due to bacteria being washed from the produce surface. Nonetheless, there was a significant reduction in presumptive *E. coli* recovered from rocket held in ECAF cold storage treatment compared to both control regimens (figure 2b), whereby a significant log reduction was observed on all sample days and a >1 log reduction was observed on all but one sample day (table 1). There was no significant difference between no treatment and RO water fog treatment regimens for recovery of presumptive Pseudomonads (figure 2c), although a significant increase in recovered Pseudomonad numbers was observed over the experimental time period, which is not unexpected given that Pseudomonads are known to colonise leafy produce (Rudi *et al.,* 2002). There was a significant log reduction in recovered presumptive Pseudomonads from rocket held in ECAF cold storage over the same time period (figure 2c), whereby the log reductions increased with increasing ECAF treatment exposure (i.e. as a factor of time), from > 0.5 log reduction on day 1 to a > 3 log reduction on day 5 (table 1). Therefore it can be concluded that ECAF is effective at significantly reducing the microbial load of both pathogenic and spoilage organisms on the surface of rocket. This is significant, given that leafy produce comprises a complex surface with a high surface area which is difficult to decontaminate (Olaimat and Holley, 2012).

Figure 3 shows the microbial recovery results on each sample day (0-5) for broccoli post-exposure to no treatment, RO water fog treatment or 1100 mV ECAF (test) treatment under cold storage conditions (4°C). There was a significant log reduction in recovered TVCs from broccoli held in ECAF cold storage compared to both control storage conditions (which were not significantly different from each other; figure 3a), whereby there was a >1 log significant reduction on all samples days (table 1). There was a significant log reduction in recovered presumptive *E.*

coli from broccoli samples held under ECAF cold storage conditions (figure 3b), whereby a >2 log significant reduction on day 1 and a >3 log significant reduction on all subsequent sample days was observed (table 1). It is therefore evident that aerosolisation is an extremely effective delivery mechanism for reducing *E. coli* on broccoli. The log reductions observed were greater than that observed within a previous study where liquid ECAS was used (Hung *et al.*, 2010), and it is possible that the aerosolisation delivery mechanism (utilising a small droplet size of 1-5 um) is a more effective at penetrating into the complex floret structure of broccoli. From figure 3c, it can be seen that there was also a significant log reduction in recovered presumptive Pseudomonads from broccoli held in ECAF cold storage compared to the controls (figure 3c) on all days except day 1 (table 1), whereby the log reduction increases with increasing ECAF treatment exposure (i.e. as a factor of time).

Figure 4 shows the microbial recovery results on each sample day (0-5) for cucumber post-exposure to no treatment, RO water fog treatment or 1100 mV ECAF (test) treatment under cooled storage conditions (14°C). There was a significant >1 log reduction in recovered TVCs from cucumbers held in ECAF cold storage compared to the controls (figure 4a; table 1), on all sampling days, except on day 5 (compared to the control water treatment). It is evident that the significant reduction in TVCs increases from day 1 to day 2, but then decreases until day 5. Although there is inherent variation in the natural microflora of test produce, the sample size (n=6) makes it likely that the observed re-growth is a real phenomenon. The cucumbers samples were cut prior to cold storage and it is known this increases the water activity and availability of nutrients to the microbial community (Olaimat and Holley, 2012). Therefore, although significant reductions on cucumbers held within ECAF storage were observed, it is evident that the microbial community was able to protect itself from the action of ECAF over a 5 day period. This is possibly mediated through

internalisation of microbes into the cut produce, which is a known protective response of food associated microorganisms (Meireles *et al.*, 2016), and could explain the observed re-growth. It cannot be discounted that there could be upregulation of microbial stress responses that enable greater protection against oxidative stress within this microbial community (Vatansever *et al.*, 2013), but this would require further experimental investigation. In contrast, there was a significant log reduction in presumptive *E. coli* recovered from cucumbers held under ECAF cooled storage (figure 4b) for the duration of ECAF treatment, whereby no presumptive *E. coli* were recovered on any sampling day after ECAF treatment had been initiated (day 1 – day 5; figure 3c and table 1). This demonstrates the significant antimicrobial potential of ECAF within cucumber cooled storage facilities. However, a consistent significant reduction in the recovered presumptive *E. coli* counts in the presence of the RO water fog treatment compared to no treatment was also observed. Similarly to the results observed for rocket, this is likely caused by *E. coli* being removed from the surface of test produce by the action of the fog, rather than direct antimicrobial action. The presumptive Pseudomonads recovered from cucumbers post exposure to no treatment, RO water fog treatment or 1100 mV ECAF (test) is shown in figure 4c. Although there is a significant difference between control regimens on day 2 to day 5, there is no consistent pattern. Further regression analysis was performed on the whole control time series data set, which showed no difference in the overall rate of change over time, whereby both samples had significantly increasing numbers of recovered presumptive Pseudomonads over the experimental time period. In contrast, there was a >1 log significant reduction in recovered presumptive Pseudomonads from cucumbers held in ECAF cold storage compared to the controls on every sample day (table 1). However, similarly to the numbers of recovered TVCs, there is a significant increase in recovered presumptive Pseudomonads from day 2 onwards (figure 4c). *P. syringae* is a known cucumber pathogen capable of replicating both within the plant and the fruit, therefore it is likely that the

physical action of cutting the cucumber samples enabled entry and replication of this organism within the fruit tissue.

Figure 5 shows the microbial recovery results on each sample day (0-5) for tomatoes post-exposure to no treatment, RO water fog treatment or 1100 mV ECAF (test) treatment under cooled storage conditions (14°C). There was no significant difference between no treatment and RO water fog treatment regimens for the total viable microbial count (TVCs) recovered from tomato samples (figure 5a), except on day 1 which is likely to be as a result of fluctuations in the natural host microflora of the produce. There was a significant reduction in TVCs recovered from tomatoes held under ECAF cooled storage conditions on all sampling days, except day 4 (compared to no treatment) and day 1 (compared to RO water fog treatment; table 1). However, it is evident that ECAF treated samples had a decreasing number of recovered TVCs over time (figure 5a), and by day 5 there is a greater than 2.5 log reduction in TVCs compared to the RO water fog treatment (table 1). Similarly to the results for cucumbers, no presumptive *E. coli* were recovered on any sampling day after ECAF treatment had been initiated (day 1 – day 5; figure 5b). However, there was a gradual decline in presumptive *E. coli* recovered from tomatoes subjected to no treatment, whereby no *E. coli* were recovered above the minimum detection limit after day 2. In contrast, presumptive *E. coli* were still recovered from the surface of tomatoes subjected to RO water fog treatment even after 5 days (figure 5b). It is therefore evident that *E. coli* were unable able to grow on the surface of tomatoes under these conditions of storage, although ECAF storage was still significantly more effective at reducing the *E. coli* load. As noted previously, the lack of recovery of any *E. coli* on day 4 for the RO water fog treatment is likely due to the action of the fog itself, rather than any inherent antimicrobial properties. In addition, there was a consistent reduction in recovered presumptive *Penicillium* spp. from tomatoes held under ECAF cooled storage conditions

compared to both controls (figure 5c). No significant log reduction was observed on day 1, but an increasing significant log reduction was observed from day 2 onwards, whereby a >3 log reduction in recovered presumptive *Penicillium* spp. was observed on day 5 (table 1). This is significant, since fungal spores are known to be more resistant to biocidal treatments (Thorn *et al.*, 2012), and it can be concluded that aerosol delivery of ECAS is effective at targeting these tenacious microbial structures on the surface of fresh produce.

The results of this study have shown that ECAS[a] when delivered as an aerosol can significantly reduce the microbial bioburden present on a range of fresh food produce. ECAS in liquid form have previously been shown to have high levels of activity when used to treat a range fresh food produce (Meireles *et al.*, 2016), including those tested within this study. For example, a 6 Log_{10} CFU g^{-1} reduction of *E. coli* has been observed when tomatoes have been treated with liquid ECAS for 5 minutes (Deza *et al.*, 2003), although the chlorine concentration applied was higher (~89 mg L^{-1} free chlorine) compared to the present study. Similarly a complete reduction in *E. coli* on artificially contaminated cucumbers (>2 Log_{10} CFU g^{-1}; below the levels of detection) has been observed when treating with liquid ECAS (32 mg L^{-1} free chlorine) for 5 minutes (Koseki *et al.*, 2004a). It is interesting to note that a complete reduction in *E. coli* was also observed within this present study when tomatoes and cucumbers were treated with ECAF, although since the first time point within this study was 24 hours, accurate determination of the initial rate of kill would require further experimentation. Liquid ECAS (~50 mg L^{-1} free chlorine) has also previously shown efficacy against *E. coli* when inoculated onto broccoli, resulting in a ~1.5 Log_{10} CFU g^{-1} reduction over 5 minutes. This present study supports this use of ECAS for controlling the microbial bioburden of broccoli, whereby significant reductions were seen when this produce type was held within an ECAF environment over 5 days. The benefit of the application of aerosolised

ECAS[a] is that it can be applied in cold/cooled storage while the produce is being held either before or during transport. However, it is essential that new technologies for fresh produce storage and microbial control do not result in any adverse effects on the quality of fresh produce. Therefore, although no adverse visual effects were recorded, future studies will focus on assessing the organoleptic properties of ECAF treated produce. In addition, these experiments were conducted at a laboratory scale. Future studies must involve industrial scale-up trials, to ensure that the levels of antimicrobial activity observed within this study are scalable within food process engineering infrastructure.

4. Conclusions

Sanitation procedures play a critical role in fresh produce safety (Olaimat and Holley, 2012) and the potential use of ECAF is only one part of the whole food production control system that could be implemented to control food spoilage and food safety organisms. This study has demonstrated that 1100 mV ECAF is capable of reducing the microbial load of both potential food spoilage and pathogenic microbes on various fresh food produce types under both cooled and cold storage conditions for a 5 day duration compared to no treatment and water fog treatment controls. These results will now be used to inform industrial scale-up trials within commercial facilities, whereby the shelf-life, microbial quality and organoleptic assessment will be performed when using this technology platform within a real food processing environment. Crucially, this will assess this microbial management system as means of controlling food spoilage, storage-life and shelf life of post-harvest produce, whilst also minimizing microbiological contamination from contact surfaces to reduce waste and improve shelf-life, food security and food safety.

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Tables

Table 1. Significant log reductions (Log_{10} CFU g^{-1}) in microbial numbers recovered from fresh food produce samples held under either ECAF cold (rocket and broccoli) or ECAF cooled (cucumber and tomatoes) storage conditions, compared to an RO water fog storage condition (n=6; ANOVA with Tukey's post hoc test). Ns = not significant; * = $p<0.05$; ** = $p<0.01$;*** = $p<0.001$.

Produce type	Microbial Recovery Method	Log reduction compared to control (water) treatment				
		Day 1	Day 2	Day 3	Day 4	Day 5
Rocket	TVCs	0.695*	0.544**	1.003**	1.635***	2.644***
	Presumptive *E. coli*	1.871***	0.839***	1.512***	1.452***	1.743***
	Presumptive Pseudomonads	0.673***	0.880***	2.635***	2.669***	3.533***
Broccoli	TVCs	1.044**	1.781**	1.289***	2.055***	1.431**
	Presumptive *E. coli*	2.141*	4.135***	4.900***	3.730***	4.204***
	Presumptive Pseudomonads	ns	1.169***	1.397***	1.681***	2.174***
Cucumber	TVCs	1.713***	2.804***	1.040***	2.890***	ns
	Presumptive *E. coli*	4.107***	2.131***	2.922***	2.339***	3.951***
	Presumptive Pseudomonads	2.827***	5.278***	2.054***	3.106***	1.430***
Tomatoes	TVCs	ns	1.062***	1.304***	1.495**	3.421***
	Presumptive *E. coli*	3.125***	2.138**	2.530**	ns	2.535**
	Presumptive *Penicillium* spp.	ns	0.993***	0.879***	1.733***	3.527***

Figure headings

Figure 1 (a) Schematic and **(b)** Photograph of the developed experimental system for delivery of ECAF. (1) RO water system. (2) Brine tank for ECAS generation. (3) ECAS generator. (4) ECAS storage tank. (5) Pizeoelectric transducer based fogging device. (6) Fogging system controller (enabling control of treatment type and duration). (7) Temperature controlled incubator.

Figure 2 (a) Total viable counts, **(b)** Presumptive *E. coli* and **(c)** Presumptive pseudomonads recovered from artificially inoculated rocket samples (25 g) removed daily from a cold storage chamber after being subjected to; (○) no treatment, (□) RO water fog or (♦) 1100 mV ECAF fogged for 8 hours a day (n=6 ± SD). MDL; Minimum detection limit.

Figure 3 (a) Total viable counts, **(b)** Presumptive *E. coli* and **(c)** Presumptive pseudomonads recovered from artificially inoculated broccoli samples (25 g) removed daily from a cold storage chamber after being subjected to; (○) no treatment, (□) RO water fog or (♦) 1100 mV ECAF fogged for 8 hours a day (n=6 ± SD). MDL; Minimum detection limit.

Figure 4 (a) Total viable counts, **(b)** Presumptive *E. coli* and **(c)** Presumptive pseudomonads recovered from artificially inoculated cucumber samples (25 g) removed daily from a cooled storage chamber after being subjected to; (○) no treatment, (□) RO water fog or (♦) 1100 mV ECAF fogged for 8 hours a day (n=6 ± SD). MDL; Minimum detection limit.

Figure 5 (a) Total viable counts, **(b)** Presumptive *E. coli* and **(c)** Presumptive *Penicillium* sp. recovered from artificially inoculated tomato samples (25 g) removed daily from a cooled storage chamber after being subjected to; (○) no treatment, (□) RO water fog or (♦) 1100 mV ECAF fogged for 8 hours a day (n=6 ± SD). MDL; Minimum detection limit.