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Gaseous ozone treatment of baby spinach within the existing production chain for inactivation of *Escherichia coli* O157:H7

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ABSTRACT

Sanitization of fresh fruits and vegetables, particularly leafy greens, is limited by penetration of sanitizers to the location of internalized pathogens. It is necessary, therefore, to adapt sanitization operations to practices in the existing produce chain. In this work, we investigated experimentally in a pilot scale, various potential sanitization options using gaseous ozone during and post vacuum cooling on the inactivation of *Escherichia coli* O157:H7 ATCC 43889. It was found that vacuum cooling causes bacterial internalization, making them harder to reach by sanitizer. However, the application of ozone during the vacuum cooling step significantly reduced ($p < 0.05$) population of viable internalized bacteria which otherwise remain unaffected by sodium hypochlorite and UV light surface treatments. The presence of free water inside the vessel available for evaporation during vacuum cooling was found to impact the microbial reduction during combined vacuum cooling and the gaseous ozone treatment. The efficacy of application of high ozone concentration (1.5 g/m^3) short term during the vacuum cooling step in combination with low ozone concentration (0.032–0.528 g/m^3) long term sanitization treatments (days) was evaluated. This combination of gaseous treatment was found more effective in microbial reduction compared to a single treatment with a strongly expressed synergistic effect. The post-treatment spinach quality evaluation, however showed an increasing degree of damage as the time of treatment increases even at low ozone concentrations.

1. Introduction

The consumption of fresh fruits and vegetables in the U.S. increased by 32% from 1982 to 1997 (Anonymous, 2001). Increased demand and variety of fresh produce, along with growing imports from countries having various standards of sanitation and worker hygiene, have resulted in an increasing number of outbreaks associated with its consumption (Tauxe et al., 1997). According to reported epidemiological data, leafy green vegetables are among the most frequently contaminated food products (Brackett, 2005; DeWaal and Bhuiya, 2007).

There are multiple ways for pathogens to contaminate fresh produce. Growing on an open field exposes products to animal and bird feces, irrigation water, fertilizer, and other potential sources of pathogens (De Roever, 1998). Irrigation or rainwater usually serves as a transport vehicle. Once on the surface, the pathogens have sufficient time for deep internalization in crevices, pores and stomata. Since it is practically impossible to control contamination of product growing on open fields, the packer/processor may have to rely on postharvest decontamination to protect consumers from foodborne illness (Eschenbach, 2007). To minimize the prevalence of foodborne disease and reduce microbial contaminations in food supplies, an effective strategy for application of sanitizers is essential (Tirpanalan et al., 2011). A number of sanitizers based on organic acids (Mendonca et al., 2004; Ortega et al., 2011; Zhao et al., 2009), chlorine (Pirovani et al., 2001; Singh et al., 2002), biocides (Knowles and Roller, 2001; Singh et al., 2002), ozone (Hunt and Marinas, 1999; Khadre et al., 2001; Singh et al., 2002; Vurma et al., 2009) and their combinations (Zhou et al., 2007) have been proposed over the years. Nevertheless, chlorine based liquid sanitizers (in the range 50–200 ppm free chlorine) still remain the most widely used for disinfection of fresh produce (Luo et al., 2011; Velázquez et al., 2009). It is commonly accepted that liquid sanitizers have difficulties in reaching bacteria embedded in small crevices and stomata. Sanitizers in gaseous form are more stable and mobile compared to the aqueous form (Guzel-Seydim et al.,

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2004; Shynkaryk et al., 2015; Vurma et al., 2009). Several gaseous sanitizers were evaluated in recent years, including ozone (Jin-Gab et al., 1999; Khadre et al., 2001), chlorine dioxide (Sun-Young et al., 2004) and cold plasma (Perni et al., 2008). Among them, ozone was reported to have the highest oxidation potential of −2.07 V (12).

Gaseous sanitizers are typically able to penetrate crevices within produce much faster than liquids, since the diffusivity of gases (~10^{-5} m^2/s) is four orders of magnitude higher than liquids (~10^{-9} m^2/s). However, sanitizers such as ozone or chlorine dioxide also are aggressive oxidizers, and may damage sensitive products such as spinach (Vurma et al., 2009). It has been shown in our previous work (Shynkaryk et al., 2015) that the rates of diffusion and reaction of sanitizer with produce are decisive in determining whether or not a given treatment is effective. For example, a high-speed wash operation is effective for dirt removal, but does not allow enough time for sanitizer diffusion to occur. However, there are various stages of fresh produce processing and handling when an application of sanitizer can be undertaken. In this work, we focus on the possibility of an early postharvest intervention for baby spinach with gaseous ozone treatment during the vacuum cooling and transportation steps. The objectives of this study were to evaluate the effectiveness of gaseous ozone for decontamination of spinach inoculated with *Escherichia coli* O157:H7, and to identify an optimal strategy for application of ozone during existing steps within the produce production chain.

2. Materials and methods

2.1. Operations in the produce chain

First, we identify the sequence of operations commonly associated with the produce chain, in justifying the choice of intervention operations. Fig. 1 shows the typical sequence of operations for leafy vegetables (information partly from Kader, 2002, and partly by direct observations). After harvest, leafy greens are typically transported for vacuum cooling, then shipped to packing sites at various locations, where they are cut, washed, centrifuged and packaged. Some potential points of intervention are also detailed in Fig. 1.

The liquid sanitizer spray step provides an opportunity to decrease initial microbial counts in leafy greens by use of a surfactant/organic acid combination. However, this operation is not within the scope of this work, and is addressed in a separate paper. The next treatment; vacuum cooling, involves the application of a vacuum to a load of vegetables, causing evaporation of surface water, and cooling by removal of the latent heat (Karel and Lund, 2003). Thereafter, the vacuum is broken, permitting air or modified atmosphere to enter the chamber. The entire process takes approximately a half-hour. The vacuum cooling operation offers an excellent opportunity for incorporation of gaseous sanitizers (Vurma et al., 2009). Since vacuum cooling is currently a widespread practice, the incorporation of a sanitizer is a modification in an existing process, and could be accomplished at lower cost than a separate stand-alone process. The next step is the transport to the packing facility, which may take up to 96 h. This is the largest window of time available for sanitizer penetration. By carefully controlled low-concentration dosing of gaseous sanitizer, it should be possible to reach deep internal crevices in produce to inactivate microorganisms that may reside within.

2.2. Bacterial strains, culture conditions and preparation of inoculum

A bacterial strain used for experiments was *E. coli* O157:H7 ATCC 43889 obtained from the culture collection of the Department of Food Science and Technology at the Ohio State University. This strain contained genes for green fluorescence protein and for ampicillin resistance, which enabled enumeration of the bacterium in the presence of the natural microbiota of baby spinach under UV light. In preparation for experiments, a loop of frozen (at − 80 °C) culture of *E. coli* was inoculated in LB broth (Difco, Becton-Dickinson, Sparks, MD) and incubated overnight at 37 °C. This was followed by another transfer into fresh LB broth for second overnight incubation. Incubated culture was harvested by centrifugation at 8000 rpm for 10 min (IEC Centra MP4R, Needham, MA) and cell pellets were resuspended in 0.1% wt/vol peptone water (Difco, Becton-Dickinson, Sparks, MD). The concentration of bacterial population was adjusted by spectrophotometric (turbidimetric) analysis (Thermo Spectronic Genesys 5 spectrophotometer, Thermo Fisher Scientific, Waltham, MA), to obtain a population of ~10^9 CFU/ml in the suspension.

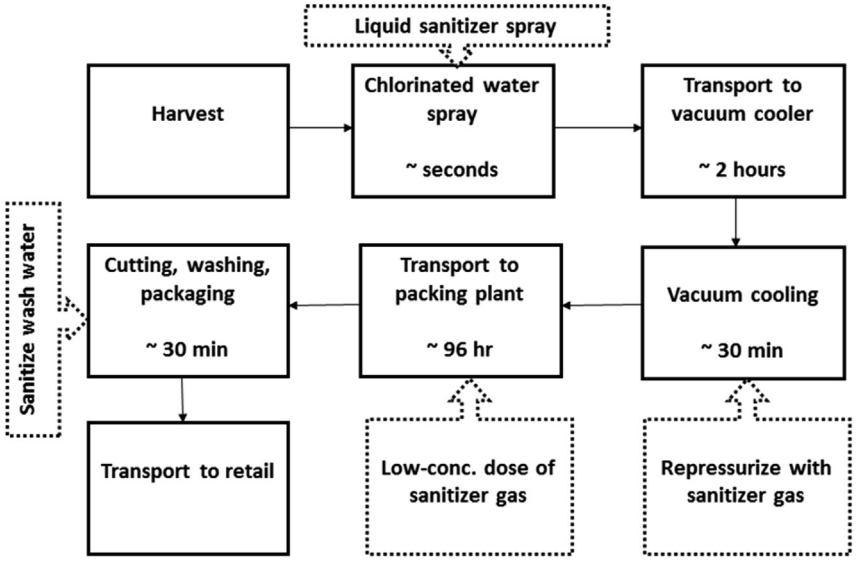

Fig. 1. Current postharvest operations for leafy vegetables, showing approximate timelines. Proposed interventions are shown by dotted arrow callouts.