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Fresh produce sanitization by combination of gaseous ozone and liquid sanitizer

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ABSTRACT

The effect of different combinations of gaseous ozone and Pro-San L against *Escherichia coli* on baby spinach was investigated. Three different ways to combine liquid and gaseous sanitizer application were compared. The most effective combination was the initial spray application of Pro-San L (0.66% Citric acid, 0.036% SDS) followed by vacuum cooling and ozonation under pressure of 68.9 kPa (10 PSIG) which reduced *E. coli* O157:H7 counts by 3.9 log CFU/g. Spray application of Pro-San L after the gaseous ozone injection under vacuum followed by immediate system pressurization to 68.9 kPa was less effective (2.7 log CFU/g reduction) due to vacuum internalization of bacteria deeper into produce tissue. This method was not significantly different from a single application of liquid sanitizer. Spray Pro-San L application followed by a long term (up to three days) gaseous ozone treatment decreased microbial load to an undetectable level after the first day of application. However the increase of exposure time to sanitizers led to some damage of spinach leaves. Long term combination liquid-gaseous sanitizer treatment resulted in better appearance of fresh produce than a single application of liquid and gaseous sanitizers.

1. Introduction

Fresh produce is widely recommended for its vitamin and nutrient content (Rickman et al., 2007). In the United States, a large portion of produce is consumed raw, and the number of foodborne outbreaks associated with these products has recently increased. Agricultural products can be exposed to microbial contamination through a variety of sources during growing, harvesting, packing, processing, shipping and preparation for consumption. The contaminated fresh produce is never heat treated to inactivate pathogens (Beuchat, 1996), (Lynch et al., 2009). Among many pathogens which cause problems with fresh produce *E. coli* O157:H7 is of particular concern because the infectious dose of this pathogen is small (less than 1000 cells) (Ackers et al., 1998; Tuttle et al., 1999). The disease can progress quickly to cause severe consequences in susceptible people, especially young children and the elderly (Cassin et al., 1998; Pai et al., 1988). Green leafy vegetables have the greatest risk of infection from manure application to soil.

While washing with water may be a useful tool for reducing potential contamination, it may also introduce or spread contaminants. To effectively eliminate or reduce pathogens from produce an application of sanitizers is necessary (Tirpanalan et al., 2011). A number of liquid and gaseous sanitizers based on organic acids (Mendonca et al., 2004; Ortega et al., 2011; Zhao et al., 2009), chlorine, chlorine dioxide (Pirovani et al., 2001; Singh et al., 2002), biocides (Knowles and Roller, 2001; Singh et al., 2002), ozone (gaseous and aqueous) (Hunt and Mariñas, 1999; Khadre et al., 2001; Singh et al., 2002; Vurma et al., 2009), (Guzel-Seydim et al., 2004; Kim et al., 1999; Rice et al., 2002) and their combinations (Zhou et al., 2007) have been used recently. Gaseous sanitizers are typically able to penetrate crevices within produce much faster than liquids, since the diffusivity of gases is four orders of magnitude higher than liquids (Shynkaryk et al., 2015). However, sanitizers such as ozone or chlorine dioxide are also aggressive oxidizers, and may, in high enough concentrations, discolor sensitive products such as spinach. Liquid sanitizers, while slow in action, and possibly ineffective in the presence of bubbles, are often more gentle, and allow for washing of dirt from produce surfaces. It would be of interest to study if combinations of liquid and gas sanitizers may be used to inactivate bacterial pathogens. In our previous work on liquid sanitizers (Pyatkovskyy et al., 2016) we compared four different liquid sanitizers (200 ppm chlorine,

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200 ppm chlorine + 0.036% sodium dodecyl sulfate [SDS], Pro San L, and 0.66% levulinic acid + 0.036% SDS). No significant differences were found between these sanitizers in terms of efficacy against *E. coli* O157:H7, thus we selected a single liquid sanitizer for study: Pro San L, a commercially prepared solution containing 0.66% citric acid, 0.036% SDS (Microcide, Inc., Detroit, MI).

Fig. 1 shows the typical sequence of operations for leafy vegetables with some potential points of intervention (Kader, 2002). 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.

Our previous work (Shynkaryk et al., 2015) has shown that because of the low diffusivities of liquid sanitizers, 15 min or greater is necessary for them to penetrate to even short distances within produce openings. Further, our more recent studies (Pyatkovskyy et al., 2016) suggested that about 30 min may need to elapse between sanitizer application and inactivation of microorganisms. This shows that produce operations that focus on high-speed operation will be ineffective for sanitization if the required time is not provided.

The liquid sanitizer spray step provides an opportunity to decrease initial microbial counts in leafy greens by use of a surfactant/organic acid combination. 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.

This step allows the possibility for incorporation of gaseous sanitizers (Vurma et al., 2009) during repressurization. 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.

The objectives of this study were to evaluate effectiveness of gaseous ozone and Pro-San L combinations for decontamination of spinach inoculated with *Escherichia coli* O157:H7 and to identify optimal strategy(ies) for application of ozone and liquid sanitizers during existing steps within the produce chain.

2. Materials and methods

2.1. Bacterial strains, culture conditions and preparation of inoculum

Bacterial strains used for experiments were *E. coli* O157:H7 ATCC 43889 and (for spray optimization experiments only) *E. coli* K12. Both were obtained from the culture collection of the Department of Food Science and Technology at The Ohio State University. The *E. coli* O157:H7 ATCC 43889 strain expressed ampicillin resistance and green fluorescence protein, which enabled selection of the bacterium in the presence of the natural microbiota of baby spinach, and its enumeration on agar plates 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 a second overnight incubation. The 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, Model 336001, Thermo Fisher Scientific, Waltham, MA), to obtain a population of ~10^9 CFU/ml in the suspension.

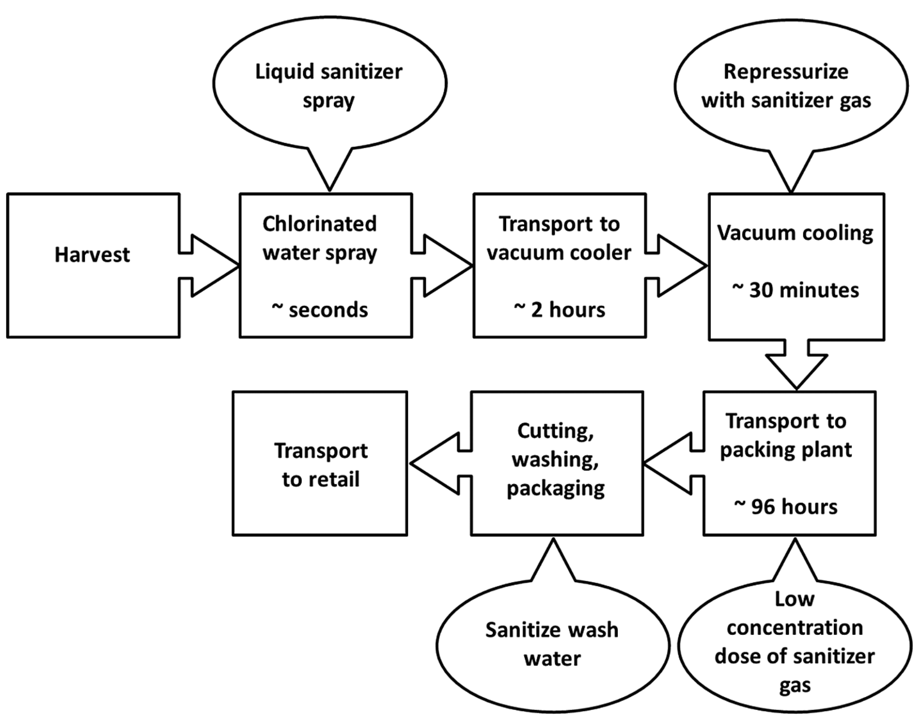

Fig. 1. Current postharvest operations for leafy vegetables, showing approximate timelines. Proposed interventions are shown by dotted arrow callouts (information partly from Kader, 2002, and partly by direct observations).