Title Development of ozone-based processes for decontamination of fresh produce to enhance safety and extend shelflife

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Abstract

Although good agricultural practices (GAP) and good handling practices (GHP) may reduce the incidence of pathogens in fresh produce, there is no known defense against contamination due to wildlife or surface water. Additionally, current strategies designed to minimize the risk of contamination during post-harvest operations of fresh leafy greens are not completely effective. Contaminations of fresh fruits with spoilage microorganisms also create economical losses by decreasing products shelflife. Therefore, it is necessary to develop sound mitigation strategies to minimize the health hazards associated with fresh produce.

The main objectives of the current study are to (i) enhance the safety of fresh produce in general, and baby spinach in particular, by integrating ozone-based sanitization steps into existing processing practices, while targeting *Escherichia coli* O157:H7 as the pathogen of concern, and (ii) to assess the feasibility of using ozone, carbon dioxide or their combinations, for reducing natural microbiota, and extending the shelf-life of strawberries.

A pilot-scale system has been successfully assembled that allows vacuum cooling and ozone treatment of fresh produce simultaneously. Combinations of vacuum cooling and ozonation (SanVac) successfully inactivated up to 2.4 log *E. coli* O157:H7 CFU/g spinach. Contribution of important treatment variables (ozone concentration, pressure and treatment time) to process lethality was also investigated using response-surface methodology. Parameters for the optimized SanVac process are of 1.5 g O_3 /kg gas-mix (935 ppm, vol/vol), 10 psig holding pressure, and 30 min holding time; these conditions achieve 1.8 log inactivation against *E. coli* O157:H7 with no apparent damage to quality of baby spinach.

A long-term, low-ozone process was also developed to treat fresh produce during transportation or temporary refrigerated storage. This treatment, termed "SanTrans", involves sparging moist gaseous ozone at 16 mg/kg (10 ppm, vol/vol) for up to 3 days. The process inactivated up to 1.4 log *E. coli* CFU/g spinach, and the optimum process resulted in 1.0 log inactivation with minimal effect on product quality.

In order to maximize inactivation on *E. coli* O157:H7, contaminated spinach was sequentially subjected to optimized SanVac (at 1.5 g/kg for 30 min and 10 psig) and SanTrans (at 16 mg/kg) processes, using freshly-harvested, unprocessed spinach that was shipped directly from California fields. These sequential treatments inactivated 4.1 to $\geq$5.0 log *E. coli* O157:H7, depending on the treatment time. Inoculated and vacuum-cooled spinach was analyzed by scanning electron microscopy. It was apparent that vacuum-cooled leaves often contained bacterial cells throughout the intercellular spaces, compared with the non-vacuum cooled leaves.

An ozone-based treatment system was designed and constructed to enhance the storage life of fresh strawberries (*Fragaria* x *ananassa*). The O_3 /CO_2 combination treatments showed synergistic effect in delaying mold growth and quality deterioration of the strawberries. When samples were treated with O_3 /CO_2 for 4 h and stored at 4°C, the initiation of visual mold appearance was delayed until the 16th day of storage; an 8-day shelflife extension, compared to untreated samples. These novel technologies are promising alternatives to conventional processes and should enhance the safety and extend the shelflife of fresh fruits and vegetables. Additionally, the new treatments should be relatively easy to integrate into existing fresh produce processes and practices.