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Reduction by gaseous ozone of *Salmonella* and microbial flora associated with fresh-cut cantaloupe

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ABSTRACT

This research investigates the efficacy of gaseous ozone, applied under partial vacuum in a controlled reaction chamber, for the elimination of *Salmonella* inoculated on melon rind. The performance of high dose, short duration treatment with gaseous ozone, in this pilot system, on the microbial and sensory quality of fresh-cut cantaloupes was also evaluated. Gaseous ozone (10,000 ppm for 30 min under vacuum) reduced viable, recoverable *Salmonella* from inoculated physiologically mature non-ripe and ripe melons with a maximum reduction of 4.2 and 2.8 log CFU/rind-disk (12.6 cm^2), respectively. The efficacy of ozone exposure was influenced by carrier matrix. *Salmonella* adhering to cantaloupe was more resistant to ozone treatment when suspended in skim-milk powder before aqueous inoculation to the rind. This indicated that organic matter interferes with the contact efficiency and resultant antimicrobial activity of gaseous ozone applied as a surface disinfectant. Conversely, in the absence of an organic carrier, *Salmonella* viability loss was greater on dry exocarp surfaces than in the wetted surfaces, during ozone treatment, achieving reductions of 2.8 and 1.4 initial log CFU/rind-disk, respectively. Gaseous ozone treatment of 5000 and 20,000 ppm for 30 min reduced total coliforms, *Pseudomonas fluorescens*, yeast and lactic acid bacteria recovery from fresh-cut cantaloupe. A dose *Ct*-value (concentration × exposure time) of 600,000 ppm min achieved maximal log CFU/melon-cube reduction, under the test conditions. Finally, fresh-cut cantaloupe treated with gaseous ozone, maintained an acceptable visual quality, aroma and firmness during 7-day storage at 5 °C. Conclusions derived from this study illustrate that gaseous ozone is an effective option to risk reduction and spoilage control of fresh and fresh-cut melon. Moreover, depending on the timing of contamination and post-contamination conditions, rapid drying combined with gaseous ozone exposure may be successful as combined or sequential disinfection steps to minimize persistence of *Salmonella* on the surface of cantaloupe melons and transference during fresh-cut processing of home preparation. Based on these results, greater efficacy would be anticipated with mature but non-ripe melons while ripe tissues reduce the efficacy of these gaseous ozone treatments, potentially by oxidative reaction with soluble refractive solids.

1. Introduction

Demand for fresh-cut fruits has been increasing in recent years, as many consumers are drawn to the convenience, portion control and conservation, and freshness as innovations in the industry have greatly improved on quality retention in packaging. However, safety concerns, especially regarding the conversion of raw fruits to a ready-to-eat (RTE) format have heightened due to increasing numbers of foodborne disease outbreaks (Beuchat, 2002; Harris et al., 2003). *Salmonella* has been isolated from fresh intact and fresh-cut fruits and vegetables including lettuce, cauliflower, cabbage, cilantro, cucumber, potato, tomato, watermelon, and cantaloupe (Saddik et al., 1985; Doyle, 1990, 2000; Tauxe, 1991; Beuchat, 1996; Harris et al., 2003). Several outbreaks of salmonellosis were associated with the consumption of bean sprouts and fresh fruits, mainly melons (Beuchat, 1996). Cantaloupe has been a vehicle for different serovars of *Salmonella enterica* (CDC, 1991; FDA, 1991; CDHS, 2000, 2001, 2002).

Potential sources of melon rind contamination include soil, contaminated irrigation water, fertilizers (non-composted manure), wildlife (e.g. birds, reptiles, amphibians), direct human contact during harvest and handling, packing house wash water,

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shipping ice, and various sources during processing and food preparation (Beuchat, 1996; Mohle-Boetani et al., 1999; Hedberg et al., 1994; Tauxe, 1997). Moreover, cross-contamination of cantaloupe can occur through inadvertent contact with raw meat (Harris et al., 2003) or human handlers during postharvest handling and packaging, transportation, distribution, final preparation at food service or in the home (Iversen et al., 1987). *Salmonella* and other foodborne pathogens attached to the external surfaces of cantaloupes are of great public health concern because they can be transferred to the edible flesh during cutting (Suslow et al., 2000; Ukuku and Sapers, 2001).

Cantaloupes produced in California, the major shipping point in the USA, have not been implicated in outbreaks of foodborne illness but the category remains on the high-priority list identified by the Food and Drug Administration (FDA) and the Canadian Food Inspection Agency (CFIA). Fruits such as cantaloupe and honeydew melon previously have been associated with 11 salmonellosis and two shigellosis outbreaks in the United States from 1973 to 2003. Reported outbreaks were associated both with whole and precut melons. More recently, during June–July 2006, a total of 41 culture-confirmed *Salmonella* serotype Oranienburg infections were diagnosed in persons in 10 northeastern US states and one Canadian province (Anonymous, 2006). The results of the investigations determined that the illness was associated with eating fruit salad in health-care facilities. Although the fruit salads were produced by one processing plant, the source of contamination was not determined but reports, based on epidemiological analysis, implicated melons. A traceback investigation indicated that cantaloupe and honeydew melons used by the processor likely originated from the United States; however, no specific farm was identified nor the association to melons definitively confirmed. However, the assumption of association to melons as the most plausible source seems fixed.

In addition, increased random surveillance triggered several non-outbreak associated recalls of cantaloupes (CFIA, 2006, 2007; FDA, 2006a, b, 2007). In the late October 2006, approximately 2560 cartons of cantaloupes were recalled following a routine sampling program test by the FDA with a positive detection of *Salmonella* bacteria. Shortly after this event, 500 cartons of cantaloupes were recalled during November 2006 coinciding with a CFIA-issued consumer advisory warning the public not to consume specific brand-labeled cantaloupes. Again, this was due to presumptive positive detection of *Salmonella* during routine surveillance sampling.

The histology of the cantaloupe rind and stem scar tissues augments attachment and penetration and might thereby reduce the effectiveness of sanitizer treatments (Richards and Beuchat, 2004). Moreover, surface hydrophobicity and both negative and positive charges are highly correlated with the strength of attachment of *Salmonella*, *Escherichia coli* O157:H7 and *Listeria monocytogenes* to the cantaloupe rind. *Salmonella* adherence is the strongest due to cantaloupe surface and that *Salmonella* has the most hydrophobic surface of these three genera (Ukuku and Fett, 2002). Therefore, microorganisms adhering to or infiltrating cantaloupe tissues is not dictated entirely by temperature differentials between fruits and immersion suspensions; rather, it is also apparently influenced by structures unique to surface tissues (Richards and Beuchat, 2004). Moreover, crevices, cracks, and small fissures in product, along with the hydrophobic nature of the waxy cuticle on the surface of many fruit and vegetables, may prevent chlorine and other sanitizers from reaching the microorganisms (Burnett and Beuchat, 2000). Therefore, preservation methods for fresh and fresh-cut cantaloupe are still under study.

Sodium hypochlorite and acidified sodium chlorite have been found effective sanitizers on inactivation of *Salmonella* or *E. coli* O157:H7 inoculated onto cantaloupes and honeydew whole melons (Park and Beuchat, 1999; Parish et al., 2003, 2005). However, on fresh-cut cantaloupe cubes, chlorine resulted in less than a 90% reduction in viable cells of the several strains of *Salmonella* (Beuchat and Ryu, 1997). Furthermore, various disadvantages are associated with chlorine-based agents such as the production of chlorinated organic compounds which are potential carcinogenics (Fawell, 2000) and can affect human and environmental safety. The concern for reactions leading to undesirable disinfection-by-products (DBP) during of post-harvest chlorination of fruits and vegetables has stimulated international interest in the development and effectiveness of new decontamination techniques including aqueous and gaseous ozone (Allende et al., 2006).

The use of ozone gas has been suggested as an alternative to reduce microbial populations on melon before cutting (Suslow, 2004a, b; Selma et al., 2008). In relative terms compared to chlorine and hypochlorite disinfectants, ozone does not create harmful. DBP residues from oxidative reaction are generally considered innocuous (Suslow, 2004a, b). Ozone solubility is limited, and therefore for post-harvest disinfection treatments gaseous ozone has been an attractive option. Also, gaseous ozone used in food processing is recognized as allowable by organic certification and regulatory bodies. Gaseous ozone is registered with the US Environmental Protection Agency (EPA) as a food contact surface sanitizer and also has an FDA acceptation and recognition approval for direct application on food products. Even though ozone is a toxic gas, it can be used safely under proper considerations when deployed by specialized generators and effective exhaust capture and destruct systems are provided. Scientists have investigated the use of ozone gas during the storage of grapes, apples and blackberries in an atmosphere containing low ozone concentrations (0.1–0.3 ppm) resulting in fungal growth reduction and an increase in their shelf life by 12th day of ozone storage (Barth et al., 1995; Bazarova, 1982; Sarig et al., 1996). Moreover, the spoilage microorganisms of vegetables such as onions, potatoes, and sugar beets are also reduced after storage in an atmosphere containing low ozone concentrations (Kim et al., 1999a, b; Gil and Selma, 2006). In contrast, doses of ozone required to rapidly kill postharvest microorganisms in a few minutes, or even hours of exposure, are very high (Smilanick, 2003). Although commercial units deliver high ozone concentrations (10,000 ppm or more) into a leak-proof chamber are available and have been used successfully for durable products such as spices, scarce information is available about the effect of these treatments on the microbial quality of the fresh-cut produce. Furthermore, ozone may also cause physiological injury of produce due to its strong oxidizing activity. For instance, bananas treated with ozone developed black spots after 8 days of exposure to 25–30 ppm gaseous ozone (Parish et al., 2003). Therefore, the possible negative impact of ozone treatment on fruit and vegetable sensory quality warrants further study (Fanchun and Wang, 2003). The objective of this study was to investigate the inactivation by gaseous ozone treatments of *Salmonella* inoculated on melon rind in presence or absence of an organic carrier matrix. We also investigated the effect of treatments on microbial and sensory quality of fresh-cut cantaloupes at two maturity stages.

2. Materials and methods

2.1. Culture preparation

Bacterial strains were obtained from Dr. Trevor Suslow Collection (PTVS) (Department of Plant Sciences, University of

California, CA, USA). A three-serotype cocktail of *Salmonella* Poona PTVS 029 (isolated from human feces, cantaloupe-associated outbreak; B. Swaminathan, CDC, Atlanta, GA, USA), Michigan PTVS 042 (isolated from human feces, tomato-associated outbreak; L.R. Beuchat, UGA) and Newport PTVS 073 (isolated from human feces, tomato-associated outbreak; B. Swaminathan) was used in this study. All isolates are uniquely coded, upon receipt or isolation, in numerical series prefaced by PTVS to designate human pathogen virulence. *Salmonella* cultures were made resistant to rifampicin (Sigma Chemical Co., St. Louis, MO, USA) by consecutive 24-h transfers of isolated colonies to tryptone soy agar (TSA; Difco, Detroit, MI, USA) with increasing concentration of rifampicin until colonies were resistant to 80 mg of rifampicin per liter. Additionally, growth curves at 37 °C were achieved in tryptone soy broth (TSB; Difco) supplemented with rifampicin (TSB-Rif) for selection of rifampicin-resistant (Rif+) bacterial colonies. Growth curves were fitted using the function of Baranyi et al. (1993) to estimate the main growth parameters, i.e. specific growth rate and lag time. After selection, Rif+ colonies were inoculated onto separate TSA plates supplemented with rifampicin (80 mg/l) (TSA-Rif) and incubated for 24 h at 37 °C. Stock bacterial cultures were kept at −70 °C in TSB-Rif containing 10% (v/v) glycerol (Sigma Chemical Co.) and subcultured twice in TSB-Rif at 37 °C for 24 h before use.

2.2. Fresh-cut processing

Unwaxed ripe (full-slip) and mature but non-ripe (half-slip) cantaloupe melons (*Cucumis melo* var. *reticulatus*) (14.0 ± 1.3 cm diameter, mean weight 900 ± 80 g) were sourced from local producers or wholesalers in California, USA, and transported under cool conditions. Melons were inspected in the laboratory and those with defects (cuts, deep abrasions, or compression bruises) were discarded. Melons were kept at 5 °C in darkness until processing the next day. For these experiments involving fresh-cut product, melons were washed by a dip in chlorinated water (150 ppm) for 5 min and vigorously scrubbed with a vegetable brush. After draining, they were manually peeled and cut into cubes (3×3 cm; 28.2 ± 5.7 g per cube) using sharp stainless steel knives. All the processing operations were conducted under sanitary conditions. Immediately after cutting, cubes were maintained at 5 °C for 30 min before sanitizing treatment with gaseous ozone.

2.3. Salmonella inoculation on cantaloupe surface

For cantaloupe rind inoculation, each culture of Rif+ *Salmonella* Poona, Michigan, and Newport was subcultured from the stock in 10 ml of sterile TSB tubes and incubated at 37 °C for 15 h until reached the stationary phase. From each culture, 0.1 ml was subcultured in 20 ml nutrient broth in flasks followed by incubation for a 15-h interval. Two milliliters of each strain (10^8 CFU/ml) were mixed together and washed three times with 0.05 M sterile phosphate buffer, pH 7, by centrifugation (Eppendorf microcentrifuge model 5415 D Brinkmann Instrument Inc., Westbury, NY, USA) at $1800 \times g$ for 10 min at 20 °C. Cell pellet was resuspended in 200 μl 0.1% peptone water. This concentrate was inoculated into 6 ml of 0.1% peptone water to prepare a 63% transmittance suspension using a Biolog 21907 turbidity meter (Biolog Inc., Hayward, CA., USA). The resulting inoculum concentration of 10^8 CFU/ml was determined by plating appropriate serial dilutions of the inoculum onto TSA-rif and incubating the plates for 24 h at 37 °C. A duplicate 6 ml, 63% transmittance sample was prepared and 0.5 ml of 10% hydrated skim milk powder (Becton Dickinson, San Jose, CA., USA) was added.

Outer rinds (exocarp) of ripe and non-ripe cantaloupes were aseptically excised from the whole melon as cores approximately 4 cm diameter by 1 cm thick to create a melon disk to conserve treatment space in the vacuum ozone chamber. These rind-disks were inoculated with 20 μl of *Salmonella* suspensions prepared either with or without addition of milk, distributed in three drops on an undamaged spot of the rind-disk to produce a final concentration of approximately 6.3 log CFU/rind-disk. After inoculation, dry spots (with and without milk) were air dried for 30 min in a biohazard safety cabinet while wet spots were not air dried. After that, dry and wet rind-disks (with and without milk added) were stored for 4 h at 5 °C before ozone treatments.

2.4. Gaseous ozone treatment

A PO-12 laboratory-scale system designed by PureOx Co. (Sparks, NV, USA), on loan to the Department of Plant Sciences (University of California) was used. The equipment consists on a stainless steel chamber, a cooling unit (to control the treatment temperature) and a bubbler assembly (to provide a humidified gas stream). The equipment provides the supply of O_2 gas to feed the ozone generator from a compressed gas cylinder. The chamber temperature, relative humidity, gaseous ozone concentration, and chamber pressure set-points or conditions were monitored from the main control panel designed by PureOx. Gaseous ozone concentration in the chamber was measured with an ozone gas analyzer (model H1-SPT, IN USA Inc., Needham, MA, USA), which allows the ozone generator to automatically switch on and off in order to maintain the target concentration. The excess of gas was neutralized by a catalytic destruct unit.

Cantaloupe rinds inoculated with *Salmonella* were treated with 10,000 ppm of gaseous ozone for 30 min (11 °C, 90–95% RH) attending to manufacture's specifications and preliminary efficacy studies on diverse fresh produce. Input from fresh-cut processors to University of California (Davis) was for high dose and short-duration treatments to allow for optimal logistical handling and throughput realities. Fresh-cut cantaloupe cubes (5 ± 1 °C) from ripe and non-ripe melons were treated for 30 min with a lower gaseous ozone concentration (5000 ppm, 11 °C, 90–95% RH) in order to find an effective ozone treatment without producing injury. Moreover, fresh-cut cantaloupe cubes from ripe melon were also treated with highly elevated doses of gaseous ozone (20,000 ppm for 30 min, 11 °C, 90–95% RH) in order to potentially observe and investigate an injury-inducing or quality-impairing threshold. For all treatments, fresh-cut cantaloupe cubes (45 cubes each; 28.2 ± 5.7 g per cube) were placed on two open-grille trays where all sides of each cube were exposed to the ozone gas with minimal support barrier interference.

All the experiments with ozone were conducted in the Mann Laboratory Biosafety Level 2 approved research space (University of California, USA) attending to strict biosafety protections and handling policies and procedures. Dual catalytic converters assured that all ozone was converted to oxygen before being discharged from the unit.

2.5. Storage

Fresh-cut ozone treated and non-treated melon cubes were packaged in polypropylene containers ($165 \times 165 \times 60$ mm) using passive modified atmosphere packaging (MAP) without initial gas injection. All samples were stored at 5 °C for up to 7 days and sensory and microbial quality was evaluated initially (before and right after ozone treatment) and after 4 and 7 days. Melon rinds inoculated with *Salmonella* and treated and non-treated with ozone were stored in air conditions at 5 °C for up to 7 days and

Salmonella counts were achieved initially (before and right after ozone treatment) and after 1, 3, and 7 days.

2.6. Microbial quality evaluation

Growth of the most important groups of microorganisms associated with the spoilage of fresh-cut melon was followed during the experiment. Five cubes of fresh-cut melon (151.6 ± 7.2 g) were transferred into stomacher bags with 100 ml of sterile buffered peptone water and vigorously rubbed and intermittently inverted for 1 min. Aliquots were taken from each bag, serially diluted in 0.1% peptone water, and surface plated on selective medium for coliforms, *E. coli*, *Pseudomonas fluorescens*, molds, yeasts, and lactic acid bacteria. Coliforms and *E. coli* were enumerated using *E. coli* and coliform chromagar (ECC; CHROMagar™, Santa Maria, CA, USA) after incubation at 37 ± 0.5 °C for 24 h. *P. fluorescens* were enumerated by King's Medium B agar (KBA; Becton-Dickinson Corporation, NJ, USA) after incubation at 25 °C for 7 days. Mold and yeast counts were performed in potato dextrose agar (PDA, Difco) plus 10% tartaric acid and 0.1 g/l streptomycin by incubation at 25 °C for 5–7 days. Lactic acid bacteria were enumerated by the standard plate count method using Man Rogosa Sharpe (MRS, Difco) agar after incubation at 25 ± 1 °C for 48 h. Microbial counts were expressed as log CFU/cube.

2.7. Recovery of inoculated Salmonella

Each inoculated cantaloupe rind sample was transferred into a stomacher bag with filter (Whirl-pak #B679 Nasco Inc., Modesto, CA., USA) and 10 ml of 0.1% sterile buffered peptone water were added. Samples were stomached (stomacher lab blender, model 80 Biomaster, Seward, UK) for 1 min. Aliquots were taken from each bag, serially diluted in 0.1% peptone water, and surface plated in duplicate on TSA-Rif. The number of colonies were counted after incubation at 37 °C for 24–48 h. Bags with wash liquid were stored at 2.5 °C for 14 h. From each sample that did not show any colonies on its respective TSA-Rif plate after 14 h, 5 ml of wash liquid were transferred to 5 ml double strength universal pre-enrichment broth (UPB; Difco) in test tubes and incubated overnight at 37 °C. A loop-full was taken from each tube and streaked on TSA-Rif. The plates were incubated at 37 °C for 24 h.

To confirm the presence–absence of viable *Salmonella* cells, a PCR method was used. Ten milliliters of wash buffer was transferring to 90 ml of UPB and incubated overnight at 37 °C. After that, 1 ml was transferred to 9 ml of tetrathionate broth base (TBB; Sigma-Aldrich Corporation, St. Louis, MO, USA) and incubated for 6–8 h at 42 ± 0.5 °C. Lastly, 1 ml of this pre-enriched sample was transferred to 10 ml of M broth (Becton Dickinson, San Jose, CA, USA) and incubated overnight at 37 °C. For DNA extraction, 1 ml aliquots were centrifuged for 10 min at $1800 \times g$. Pellets were washed three times with Tris–EDTA 1 × solution pH 8 (Cole-Parmer Instrument Company, IL, USA) by centrifugation at $1800 \times g$ for 10 min and resuspended in 50 μl of sterile nanopure water. Next boiling (95 °C for 10 min) lysed cells to release the DNA. Samples were cooled on ice for 10 min. After extraction with the DNeasy Tissue kit (Qiagen, Valencia, CA, USA), DNA was eluted in 100 μl bi-distilled water and 5 μl were used as template for amplification. A PCR reaction was run using *Salmonella* specific primers INVA-1 (ACAGTGCTCGTTTACGACCTGAAT) and INVA-2 (AGACGACTGGTACTGATCGATAAT), as described previously (Chiu and Ou, 1996). Briefly, PCR reaction were performed in 50 μl as final volume, containing 5 μl of 10 × PCR amplification buffer, 1.5 mM $MgCl_2$, 200 μM (each) the four deoxyribonucleoside triphosphates, 1 mM (each) of primer pair INVA1/INVA2, 1.25 U of Taq polymerase (Promega, Road Madison, WI, USA). Amplification conditions were: 5 min at 94 °C, 35 cycles of 30 s at 94 °C, annealing of primers for 30 s at 56 °C and primer extension for 2 min at 72 °C and a final extension of 10 min at 72 °C. Reactions were carried out in a GeneAmp PCR System 9700 (PE Applied Biosystems, Norwalk, CT, USA) thermal cycler. PCR products were electrophoresed through 2% agarose gel in TAE buffer (40 mmol/l Tris–acetate, pH 8.0; 1 mmol/l Na_2EDTA). After staining with ethidium bromide, the amplified DNA fragments in the gel were visualized and photographed under UV illumination.

2.8. Sensory evaluation

The organoleptic characteristics including visual quality, aroma, texture and translucency of non-inoculated, fresh-cut melon cubes were evaluated initially and after 4 and 7 days of storage by a four-member expert panel. Visual quality was scored on a 9–1 scale, where 9 = excellent and 1 = unusable/unacceptable. Aroma and texture were scored based on a scale of 5–1, where 5 = full characteristic or fresh and 1 = complete lacking or soft, respectively. Translucency was scored on a 1–5 scale, where 1 = none and 5 = severe.

2.9. Firmness, color changes and soluble solid content

Firmness of fresh-cut melon was carried out by using a TA-XT Texture Analyzer (Texture Technologies Corp. Scarsdale, NY, USA). This test was based on the resistance of the center side of each melon piece to pressure by a puncture test, using a round-tipped cylindrical 11 mm diameter probe (1 mm/s to a depth of 5 mm). Color changes of fresh-cut melon cubes were analyzed on the pulp surface with a Minolta Chroma Meter CR 300 (Minolta Co. Ltd., Osaka, Japan). Soluble solid (SS) content was measured using a digital temperature-compensated Antago N1 handheld refractometer (Tokyo, Japan).

2.10. Statistical analysis

There were three separated experiments per treatment. Microbial data represent the mean of the three experiments, each consisting of triplicate samples (5 cubes each) for each treatment and sampling date. Sensory data represent the mean of the three experiments with three replicates per each treatment and evaluation period. Each replicate consists of 10 melon cubes. Analysis of variance (ANOVA), followed by Tukey's method with a significance level of $P \leqslant 0.05$ was carried out on these data using SPSS (Windows 2000, Statistical Analysis program) (Visauta, 2002).

3. Results and discussion

3.1. Effect of ozone on microbial stability of fresh-cut melon

Initially, microbial population of ripe control (untreated ripe melon) averaged 2.7, 2.9, 4.4, and 3.9 log CFU/cube for coliforms, *P. fluorescens*, yeasts, and lactic acid bacteria, respectively (Fig. 1). *E. coli* and molds were not recovered from selected fresh-cut melons. There were significant differences ($P \leqslant 0.05$) between the ripe and non-ripe control (untreated non-ripe melon) except for lactic acid bacteria. Initial microbial counts on the non-ripe control were 0.5–0.7 log CFU/cube units lower than in ripe control except for lactic acid bacteria.

The tested ozone treatments (5000 and 20,000 ppm for 30 min) reduced initial counts of all microbial groups tested in

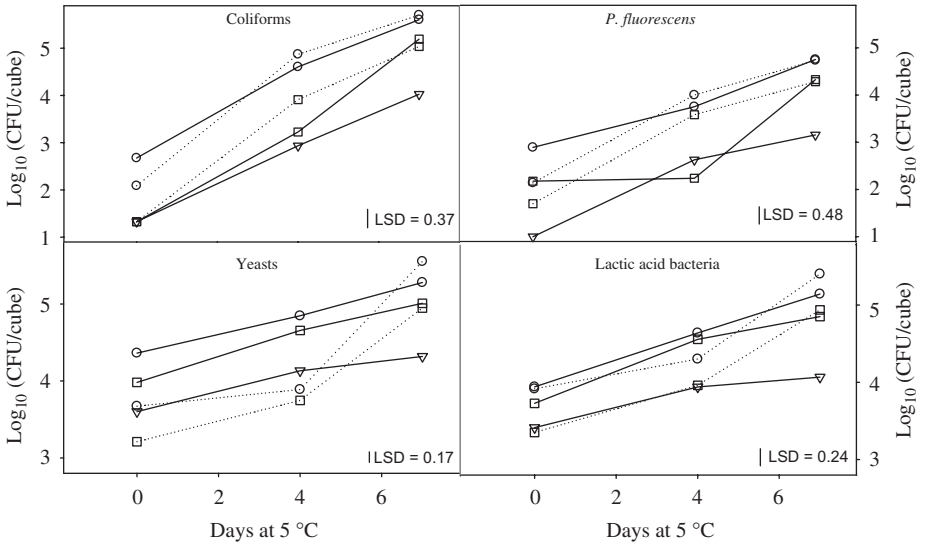

Fig. 1. Effect of gaseous ozone on growth of coliforms, *P. fluorescens*, yeasts and lactic acid bacteria (log CFU/cube) on fresh-cut melon during 7-days storage at 5 °C. Control non-treated (○), 5000 ppm/30 min (□), 20,000 ppm/30 min (▽), ripe melon (—), non-ripe melon (···). Values are means of three experiments with triplicate determinations per experiment. Bars represent LSD at $P \leqslant 0.05$.

relation to untreated samples (Fig. 1). In ripe and non-ripe melons, 5000 ppm/30 min ozone treatment reduced initial microbial counts by 1.1 ± 0.4, 0.5 ± 0.2, 0.4 ± 0.3, and 0.5 ± 0.1 log CFU/cube reductions for coliforms, *P. fluorescens*, yeasts and lactic acid bacteria, respectively. No significant differences were found in ripe and non-ripe melons treated with 5000 ppm/30 min ozone except for yeasts and lactic acid bacteria. However, at the end of the storage, lactic acid bacteria in ripe and non-ripe melons treated with 5000 ppm/30 min ozone achieved similar levels (Fig. 1). Therefore, ozone treatment was effective either on non-ripe and ripe melons achieving a higher control of yeasts on non-ripe melons. Delay in the growth of yeasts on fresh-cut melon is an important factor as they are one of the main determinants of end of shelf-life on fresh-cut fruits.

The efficacy of 20,000 ppm/30 min ozone treatment on microbial inactivation was somewhat improved as compared to 5000 ppm/30 min ozone treatment being especially more effective on *P. fluorescens* (Fig. 1). Thus, 20,000 ppm/30 min ozone treatment reduced initial *P. fluorescens* counts by 1.9 log CFU/cube, whereas 5000 ppm/30 min ozone treatment reduced *P. fluorescens* counts by 0.5 log CFU/cube. *P. fluorescens* reduction may be beneficial to quality retention due to this microorganism's contribution to spoilage of perishable, refrigerated product. At the end of the storage, 20,000 ppm/30 min ozone treatment was most effective in slowing down microbial growth of total coliform, *P. fluorescens*, yeast and lactic acid bacteria counts being 1.6, 1.6, 0.7, and 1.1 log CFU/cube units, respectively, lower than on untreated samples. Neither level of ozone *Ct*-value (concentration × exposure time) completely eliminated the different microbial groups from fresh-cut melon. It is widely accepted that this is not a desirable outcome as total sterility might leave fresh produce at higher risk of bacterial pathogen growth in the event of re-contamination. It has been also reported that the presence of competing microorganisms contributes to the reduction of pathogens (Liao and Fett, 2001). In fact, Carlin et al. (1996) reported that reducing microflora on salad leaves by a chemical disinfection permitted, in some cases, a better growth of *L. monocytogenes*. Therefore, it is important to recognize that techniques aimed at decreasing the activity of spoilage microorganisms might possibly enhance the growth of pathogens due to a reduction in competing flora and phytotoxic injury to plant tissues (Parish et al., 2003).

3.2. Effect of ozone on sensory quality of fresh-cut melon

Initially, the visual quality of fresh-cut melon was excellent after ozone treatments and aroma, firmness and SS% were not significantly different from untreated samples (Table 1). However, translucency and L^* parameters were slightly increased and decreased, respectively, after ozone treatments. During storage, samples treated with ozone maintained a good visual quality, aroma and firmness. SS was maintained during storage without significant differences between treated and untreated samples (Table 1). However, when the storage life was prolonged, a slight increase in translucency was observed being more evident in samples treated with the higher ozone dose. At the end of the storage, treated and untreated samples maintained the full typical aroma, color and a very firm and turgid texture. Furthermore, neither 5000 nor 20,000 ppm gaseous ozone treatment notably affected the global sensory quality (visual quality, aroma, color and texture) of fresh-cut melon during storage. Therefore, gaseous ozone treatment delivered at high *Ct*-value, achievable in a vacuum chamber system, of fresh-cut melon could be an alternative method for slowing down the growth of pathogen and spoilage microorganisms without considerably affecting its sensory quality.

3.3. Ozone effect on Salmonella inoculated on cantaloupe rind

The efficacy of gaseous ozone in reducing the population of *Salmonella* inoculated on cantaloupe rind and stored for 7 days is shown in Fig. 2. Initial *Salmonella* population of inoculated cantaloupes was 6.3 log CFU/rind-disk for dry spots (with and without milk added), and 5.0 and 5.8 log CFU/rind-disk for wet spots (with and without milk added, respectively). Initial microbial inactivation by ozone (10,000 ppm/30 min) was higher on non-ripe than ripe melons achieving 3.8 and 2.8 log CFU/rind-disk reductions on dry spots, respectively (Fig. 2). Reduction of *Salmonella* levels for up to 7 days of storage was also greater on non-ripe melons. These results could indicate that ripe melon exocarp tissue may have more accessible protected spaces or allows better post-arrival bacterial aggregation (Richards and Beuchat, 2004), which would cause incomplete ozone penetration to the cells remaining on the surface for an extended time under

Table 1
Sensory evaluation of fresh-cut melon cubes treated 30 min with gaseous ozone and stored for 7 days at 5 °C

Treatment	Storage (days)	Visual quality[a]	Aroma[b]	Translucency[c]	L^*	Firmness[d]	SS (%)
Untreated	0	8.7a	3.9a	1.3c	59.9a	10.9a	10.9a
	4	7.9c	4.0a	2.0b	59.1a	10.0a	11.0a
	7	7.8c	3.9a	2.1b	59.4a	10.4a	10.5b
5000 ppm O_3	0	8.3b	3.9a	1.7b	58.4b	11.6a	11.0a
	4	7.9c	3.7a	2.1b	56.7c	11.0a	11.0a
	7	7.2d	3.3a	2.8a	56.1c	11.3a	11.1a
20,000 ppm O_3	0	8.2b	3.9a	1.8b	57.9b	11.8a	11.1a
	4	7.9c	4.0a	2.1b	55.7c	11.3a	11.3a
	7	7.1d	3.6a	2.9a	54.7c	10.8a	11.0a

Data within the same column followed by different letter are significantly different at ($P \leqslant 0.05$).
[a] Scale 9–1, where 9 = excellent, 7 = good, 5 = fair, 3 = poor, 1 = unusable/unacceptable.
[b] Scale 1–5, where 1 = none, 2 = slight, 3 = moderate, 4 = moderate/severe, 5 = full characteristic.
[c] Scale 1–5, where 1 = none, 2 = slight, 3 = moderate, 4 = moderate/severe, 5 = severe.
[d] Firmness of fresh-cut melon was measured as resistance of the center side of each melon piece to pressure and expressed as Newton (N).

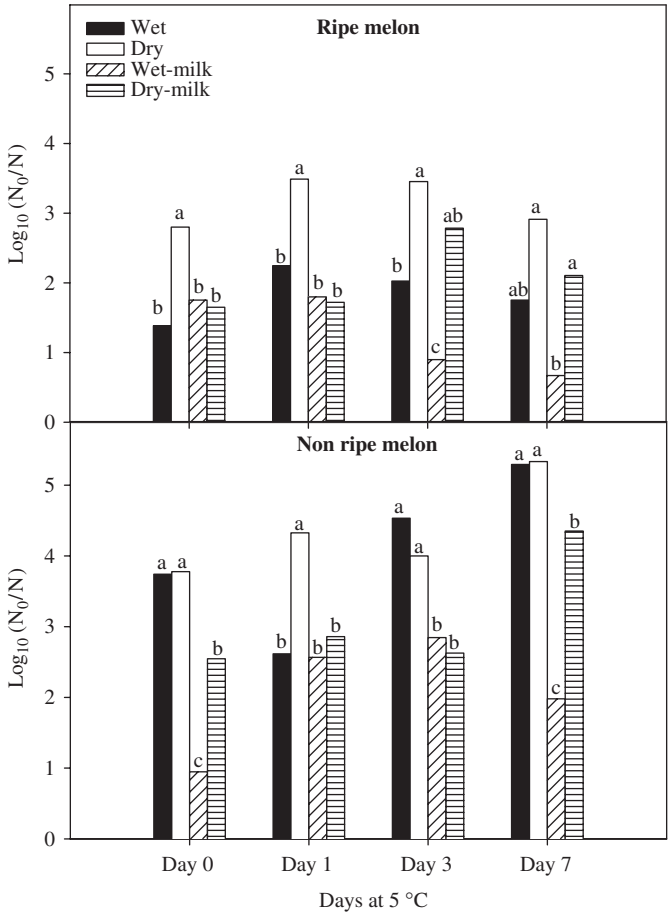

Fig. 2. Effect of gaseous ozone (10,000 ppm O_3/30 min) on reduction of *Salmonella* inoculated on melon rind initially and during storage 7 days at 5 °C. Reduction values ($\log_{10} N_0/N$) represent *Salmonella* counts in melon rind samples treated with ozone (N) in relation to *Salmonella* counts in untreated samples (N_0) at the different sampling dates. Values are means of three experiments with triplicate determinations per experiment. Means not followed by the same letters are significantly different ($P<0.05$) from each other.

refrigerated storage. Furthermore, the less waxy surface and availability of nutrients, even within the netted-rind of ripe cantaloupe, would be expected to enhance the survival of *Salmonella*, even at refrigeration temperatures as it has been indicated previously (Richards and Beuchat, 2004).

Ozone treatment was effective initially and during 7 days of storage at 5 °C while *Salmonella* levels of untreated samples were not reduced through 7-day storage. A previous study has indicated that *Salmonella*, *Shigella* and *E. coli* O157:H7 can survive on the surface of cantaloupe under stressful environmental conditions such as temperature (Suslow, 2004a, b). This inactivation on treated samples through 7 days at 5 °C could indicate the existence of a sublethal damage on treated cells, which were finally inactivated during 5 °C storage. Some researchers have indicated that viability of *E. coli* was unaffected by short-term exposure to ozone gas but membrane permeability was compromised (Komanapalli and Lau, 1996). Therefore, a synergistic effect of ozone treatment and low refrigeration temperature could have produced these results.

On the other hand, in ripe melons, inactivation was higher in dry than in wet spots achieving 2.8 and 1.4 initial log CFU/rind-disk reductions, respectively (Fig. 2). This greater level of reduction on dry spots was maintained during storage. This result provides support for the functionality of rapid melon rind drying as one obstacle to persistence of *Salmonella* on the surface of cantaloupe melons and it is in agreement with a previous research (Lang et al., 2004).

Lastly, the effect of powdered milk, a potentially interfering organic substrate, on the antimicrobial activity of gaseous ozone was investigated. Previous studies have indicated that there are many factors, including the composition of the inoculum carrier (water or a high organic matrix) used to prepare inocula and inoculation method, could affect survival, growth and retrieval of *Salmonella* on intact or wounded cantaloupe rind (Beuchat et al., 2001; Beuchat and Scouten, 2004). Thus, consideration of these observations is recommended when developing a method to test the efficacy of sanitizers in killing salmonellae on the rind surface of inoculated cantaloupes and to detect or enumerate salmonellae that may be natural contaminants (Beuchat and Scouten, 2004). Furthermore, skim milk and bovine serum albumin are often used to evaluate the influence of organic matter on the antimicrobial activity of disinfectants and antiseptics for use in medical, food hygiene and veterinary areas (Rosenthal et al., 1982; Gelinas and Goulet, 1983; Hammer et al., 1999), according to the three levels of testing proposed by the European committee (EN1276, 1997). Inoculum prepared with milk (wet-milk and dry-milk spots) and added to cantaloupe rind-disks were more resistant to ozone treatment achieving a lower initial inactivation of *Samonella* in relation to dry spots without milk addition (Fig. 2). This indicated that organic matter such as milk could protect bacteria against gaseous ozone treatment. Moreover, ozone could react with organic matter of melon surface neutralizing some of the ozone

before it reaches microbial cells, thereby reducing its effectiveness. Therefore, the efficacy of ozone treatment would likely be strongly influenced by any organic matrix that contains the contaminating pathogens or organic matter deposited on the melon surface. During 7-day storage at 5 °C, the inactivation level of *Salmonella* on wet-milk and dry-milk spots was also inferior to dry spots without milk added. Clearly, the level of survival and persistence of bacteria was dependent of the added suspending organic matrix. This is in agreement with previous works (Kim et al., 1999a, b; Selma et al., 2006) where it was indicated that susceptibility of microorganisms to ozone varies with the presence of additives such as acids, surfactants, and sugars.

After 7-day storage, 5.4 log CFU/rind-disk reductions of *Salmonella* from non-ripe melons with dry surface were achieved in relation to untreated samples (Fig. 2). Furthermore, *Salmonella* viable cells were not detected from non-ripe melons with dry surface even after non-selective pre-enrichment and sensitive PCR techniques (Fig. 3). Therefore, if contamination occurred close to or during harvest, rapid drying could be one of the several potential barriers to persistence of *Salmonella* on the surface of cantaloupes melons and further inactivation might be predicted by treatment with high doses of gaseous ozone in a contained chamber.

Many strategies for single, combined, or sequential disinfection treatments have been studied or suggested to achieve desirable levels of pathogen reduction on fresh and fresh-cut produce. Gaseous ozone has been identified in the commercial produce sector as an ideal sanitizing agent, especially for field packed commodities that are not washed or cannot tolerate wetting prior to shipping due to superficial mold growth or accelerated decay. Commercial systems for vacuum ozone 'fumigation' of trailer-load quantities of palletized durable goods are already in use. The possibility of high-dose treatments of palletized loads of vented fiberboard cartons or high air-flow returnable plastic containers of produce was of interest among commercial handlers. For fresh-cut applications, the absence of deleterious treatment residues and low risk of undesirable DBP makes ozone an attractive treatment that has received much empirical attention and claims of great benefit. The outcomes of these studies, within the limitations of the pilot-scale ozone fumigation chamber and the experimental design, reveal both the potential benefits of existing large-scale gaseous ozone treatment systems with cantaloupe and some clear uncertainties in commercial applications. While reductions in both applied and indigenous microbial populations were achieved, various factors interfered with the realization of efficacy that was demonstrated in some system conditions. As we are unaware of any studies that clearly elucidate the nature and physiologic condition of naturally contaminating *Salmonella* on intact cantaloupe, the predictive value of artificial decontamination studies may be fairly questioned. The impact of organic matrices on tolerance of contaminating *Salmonella* to doses of 10,000 ppm ozone is particularly concerning.

In addition to potential limitations of gaseous disinfection due to fruit-to-fruit and fruit-to-container contact points, the characterization of potential negative impacts on product quality that we are lacking is prior to this study. We feel that the significance of this work is to provide a cautionary background for those in commercial development or application of this technology to extend these findings to expanded studies under operational scales.

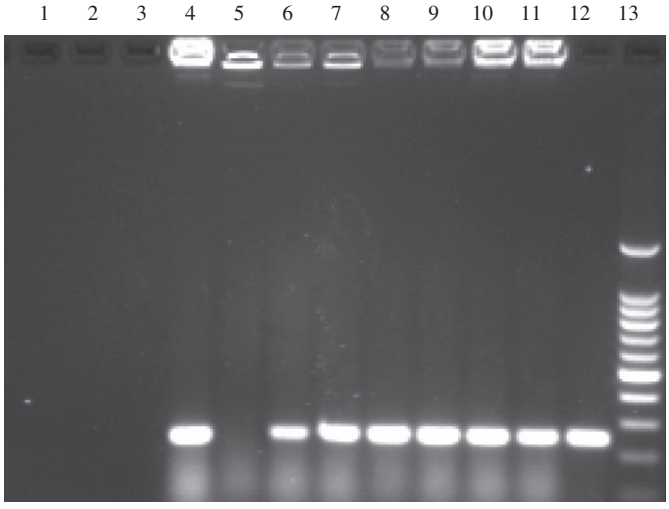

Fig. 3. *Salmonella* PCR from melon rinds inoculated with *Salmonella* and treated with ozone. (1) Negative control; *treated with 10,000 ppm O_3/30 min*: (2, 3) dry-milk inoculum, (4) wet-milk inoculum, (5) dry inoculum, (6–7) wet inoculum; *non-treated with ozone*: (8) dry-milk inoculum, (9) wet-milk inoculum, (10) dry inoculum, (11) wet inoculum, (12) positive control, (13) ladder.

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