# Efficacy of Gaseous Chlorine Dioxide as a Sanitizer for Killing *Salmonella,* Yeasts, and Molds on Blueberries, Strawberries, and Raspberries

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### ABSTRACT

Gaseous chlorine dioxide (ClO<sub>2</sub>) was tested for its effectiveness in killing *Salmonella*, yeasts, and molds on blueberries, strawberries, and red raspberries. An inoculum (100  $\mu$ l, 6.0 to 6.8 log CFU/g of fruit) that contained five serotypes of *Salmonella enterica* was deposited on the skin, calyx tissue, or stem scar tissue of blueberries, skin or stem scar tissue of strawberries, and skin of red raspberries, dried for 2 h at 22°C, then held for 20 h at 4°C and 2 h at 22°C before treatment. Sachets that contained reactant chemicals were formulated to release gaseous ClO<sub>2</sub> at concentrations of 4.1, 6.2, and 8.0 mg/ liter of air within treatment times of 30, 60, and 120 min, respectively, at 23 ± 1°C. Lethality of ClO<sub>2</sub> to *Salmonella*, yeasts, and molds was measured when fruits were in an atmosphere that contained 75 to 90% relative humidity. Treatment with 8.0 mg/liter of ClO<sub>2</sub> significantly ( $\alpha = 0.05$ ) reduced the population of *Salmonella* on blueberries by 2.4 to 3.7 log CFU/g. Lethality was higher to cells in inoculum placed on the skin compared with the stem scar tissue. Populations of *Salmonella* on strawberries treated with 8.0 mg/liter of ClO<sub>2</sub> were reduced by 3.8 to 4.4 log CFU/g; a significant reduction of 1.5 log CFU/g of raspberries was achieved. Treatment with 4.1 to 8.0 mg/liter of ClO<sub>2</sub> caused reductions in populations of yeast and molds on blueberries, strawberries, and raspberries of 1.4 to 2.5, 1.4 to 4.2, and 2.6 to 3.0 log CFU/g, respectively. Treatment with 4.1 mg/liter of ClO<sub>2</sub> did not markedly affect the sensory quality of fruits stored for up to 10 days at 8°C. Results indicate that gaseous ClO<sub>2</sub> has promise as a sanitizer for small fruits.

During the past several years, the per capita consumption of fresh produce in the United States has increased. This has undoubtedly contributed, in part, to a higher number of cases of foodborne illness associated with fruits and vegetables. Small fruits are among the produce that have been linked to outbreaks of enteric infections. Strawberries have been implicated in outbreaks of hepatitis A (32), and raspberries have been associated with outbreaks of *Cyclospora cayetanensis* infection (28). Salmonella and Listeria monocytogenes can survive on produce throughout their shelf life (3, 6, 15, 29).

Raw fruits and vegetables are occasionally contaminated with foodborne pathogens; however, there is a general lack of efficacy of sanitizers in killing or removing these pathogens (5). This can be partially attributed to difficulties in delivering aqueous chemical sanitizers to areas on the surface of produce in which pathogens may be lodged (8). Treatment with aqueous chemical solutions can also leave residual moisture on fruits and vegetables, which can promote the growth of molds. Infection of produce with molds can in turn increase the pH of tissues and enhance the growth of *Salmonella (44), Escherichia coli* O157:H7 (*35),* and *Clostridium botulinum (13)*.

Sanitizers such as gaseous chlorine dioxide  $(ClO_2)$  have been explored as alternatives to aqueous chemicals for

sanitizing fruits and vegetables eaten raw. Gaseous  $ClO_2$  has some advantages over chlorinated water in that it can break down phenolic compounds and remove phenolic tastes and odors from the water, does not react with ammonia, and has 2.5 times the oxidation capacity of chlorine; its bactericidal efficacy is not markedly affected by pH and it has greater sporicidal activity (9, 16, 33). Loss in permeability control with nonspecific oxidative damage of the outer membrane and inhibition of respiration are among the events associated with lethality of  $ClO_2$  to vegetative bacterial cells (4).

Several studies have shown gaseous  $ClO_2$  to be effective in killing enteric pathogens on fruits and vegetables. Treatment of uninjured green peppers with gaseous  $ClO_2$  at a concentration of 3 mg/liter reduced the number of *L. monocytogenes* by more than 6 log CFU/5 g (23). Treatment of peppers with 0.6 mg/liter of gaseous  $ClO_2$  for 30 min at 22°C and 90 to 95% relative humidity caused a 7.3-log CFU/5 g reduction of *E. coli* O157:H7 on the uninjured surface (22). Treatment of cut apples with gaseous  $ClO_2$  at a concentration of 3.3 mg/liter for 20 min resulted in a reduction of *E. coli* O157:H7 of 5.9 log CFU/5 g (14). Sapers et al. (39) reported that the number of nonpathogenic *E. coli* inoculated onto apples was reduced by 4.5 log CFU/g, with minimal quality loss, by treatment with gaseous  $ClO_2$  at 0.3 mg/liter.

Gaseous  $ClO_2$  (0.5 to 2.0 ppmv/g of material) was used

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to effectively control the spread of molds in libraries (45). Although the fungicidal effect was demonstrated on books, gaseous ClO<sub>2</sub> also has potential as a sanitizer for reducing yeast and mold populations in food processing plants and on fresh fruits and vegetables. Inoculation of epoxy-coated stainless steel strips identical to those used in juice tanks with Eurotium, Penicillium, Candida, and Saccharomyces *cerevisiae* at populations of  $>4 \log CFU$  per area (2.5 by 7 cm), followed by gaseous ClO<sub>2</sub> treatment, resulted in reductions to populations below detectable limits (19). Other gaseous chemicals have shown promise as sanitizers for fruits and vegetables. Treatment of fruits (40), mung bean seed (12), and alfalfa seed (46) with gaseous acetic acid and apples (39) and prunes (41) with vapor-phase hydrogen peroxide has been reported to reduce microbial populations and extend shelf life.

The efficacy of gaseous  $ClO_2$  gas in killing *Salmonella*, yeasts, and molds on small fruits has not been reported. The objective of this study was to evaluate gaseous  $ClO_2$  for its effectiveness in killing *Salmonella* inoculated onto the surface of blueberries, strawberries, and red raspberries. Inactivation of yeasts and molds that occur naturally on the fruits was also determined.

## MATERIALS AND METHODS

**Bacteria used and maintenance of cultures.** Serotypes of *Salmonella enterica* isolated from alfalfa sprouts (serotype Agona), feces of patients in tomato-associated outbreaks of salmonellosis (serotypes Baildon and Montevideo), orange juice (serotype Gaminara), and a cantaloupe-associated outbreak (serotype Michigan) were used. All serotypes were grown in tryptic soy broth (Difco, Becton Dickinson, Sparks, Md.) supplemented with nalidixic acid (Sigma, St. Louis, Mo.) (TSBN) at a concentration of 50  $\mu$ g/ml at 37°C for 24 h. Cultures were combined with glycerol (85:15, vol/vol, culture:glycerol) and stored at -30°C until used.

Preparation of inoculum. Frozen cell suspensions of the five S. enterica serotypes were thawed and streaked onto tryptic soy agar (Difco, Becton Dickinson) supplemented with nalidixic acid (50 µg/ml) and sodium pyruvate (1 g/liter) (TSANP). The TSANP plates were incubated at 37°C for 24 h before picking colonies to be transferred into 10 ml of TSBN. Tubes were incubated at 37°C for 24 h. A minimum of two consecutive 24-h transfers were made via loop inoculum (ca. 10 µl) into 10 ml of TSBN before cells were harvested by centrifugation at 2,000 imesg for 15 min (Centra CL2 centrifuge, International Equipment Co., Needham Heights, Mass.). The supernatant was decanted and cells were resuspended in 5 ml of sterile 5% horse serum (1:9, vol/vol, deionized water:horse serum) (Sigma). Suspensions of each serotype were combined to give 20 ml of a five-serotype mixture of S. enterica that contained approximately equal populations (9 log CFU/ml) of each serotype. Populations were determined by serially diluting suspensions in sterile 0.1% peptone and surface plating duplicate 0.1-ml samples on TSANP. Plates were incubated at 37°C for 24 h before colonies were counted.

**Produce tested.** Blueberries (*Vaccinium corymbosum* L.), strawberries (*Fragaria ananassa* Duchesne), and red raspberries (*Rubus idaeus* L.) were purchased at a local produce market in Griffin, Ga., and stored at 4°C for a maximum of 2 days before use in experiments. Before inoculation with *Salmonella*, the fruits were adjusted to  $22 \pm 1$ °C during a 1- to 2-h period. Samples

that consisted of 12 blueberries  $(20 \pm 1 \text{ g})$ , 5 strawberries  $(100 \pm 10 \text{ g})$ , or 6 raspberries  $(20 \pm 1 \text{ g})$ , all free of visible wounds, cuts, and bruises, were placed in single layers on plastic trays (14 cm long by 14 cm wide by 2.5 cm high) in preparation for inoculation.

**Inoculation of berries.** Blueberries, strawberries, and raspberries at  $22 \pm 1^{\circ}$ C in plastic trays were spot inoculated with 100  $\mu$ l of a five-serotype mixture of *S. enterica* using a micropipettor. Inoculum was deposited on the skin, calyx tissue, or stem scar tissue of blueberries; separate samples were used for each inoculum site. Inoculum was applied on either the skin or stem scar area of separate samples of strawberries. Only the external skin surface of raspberries was inoculated.

To prevent inoculum from running off the sides of fruits and to facilitate drying, small approximately equal volumes of inoculum were applied to several berries in the same sample or up to five locations at each site on the same berry. All fruits were inoculated in a biosafety hood and dried for 2 h at  $22 \pm 2^{\circ}$ C, followed by storing in plastic containers at 62% relative humidity for 20 h at 4°C. Before treatment with gaseous ClO<sub>2</sub>, fruits were placed in a biosafety hood for 2 h at  $22 \pm 2^{\circ}$ C.

Relative humidity. Nine samples of blueberries (three samples with the skin inoculated, three with the calyx tissue inoculated, and three with the stem scar tissue inoculated), six samples of strawberries (three with the skin inoculated and three with the stem scar area inoculated), or three samples of raspberries with the skin inoculated were placed in a Fisherbrand transparent Plexiglas desiccator cabinet (45.7 cm high by 30.5 cm wide by 30.5 cm long; 31.1-liter volume; Fisher Scientific, Pittsburgh, Pa.). Samples were placed on the bottom three shelves of the four-shelf cabinet. High (75 to 90%) relative humidity was achieved by placing 20 ml of hot water (initially at 97 to 99°C) in a shallow plastic dish (8.6 by 8.6 by 2.2 cm) on the bottom shelf during treatment of berries with ClO<sub>2</sub>. A brushless 12VDC cooling fan (6.9 by 6.9 by 2.5 cm) (RadioShack, Fort Worth, Tex.) was strategically placed on each of the four shelves to circulate the air. A Fisher Scientific Thermo-Hygro recorder (model no. 11-661-13) was used to monitor relative humidity and temperature inside the treatment cabinet.

**Gaseous CIO<sub>2</sub> treatment.** Fruits inoculated with approximately 6.0 to 6.5 log CFU/g of *Salmonella* were placed in the cabinet and treated with air (control) or gaseous CIO<sub>2</sub> for 0, 30, 60, and 120 min. Sachets (ca. 9 by 18 cm) that consisted of two compartments, one that contained a granular porous solid impregnated with sodium chlorite and the other that contained a granular porous solid impregnated with acid and an acid precursor (ferric chloride), were supplied by ICA TriNova, Inc., Marietta, Ga. Breakage of the septum between the two compartments, followed by mixing the chemicals, initiated the production of CIO<sub>2</sub> gas. The mixture of chemicals in three sachets was formulated to release gaseous CIO<sub>2</sub> into the cabinet at concentrations of 4.1, 6.2, and 8.0 mg/liter within 30, 60, and 120 min, respectively, at 23  $\pm$  1°C.

The gaseous  $ClO_2$  concentrations released into the treatment chamber can also be defined as ppmv, since a gas phase concentration of 1 mg/liter is equivalent to 362 ppmv. An alternative way to report concentrations of 4.1, 6.2, and 8.0 mg/liter of gaseous  $ClO_2$  released into the treatment chamber is 1,484, 2,244, and 2,896 ppmv in 30, 60, and 120 min, respectively. Concentrations of  $ClO_2$  released during a 2-h period are shown in Figures 1 and 2. Concentrations of  $ClO_2$  were determined by titrating the amount of iodine formed by its reaction with potassium iodide





FIGURE 1. Atmospheric relative humidity (%;  $\blacksquare$ ) and chlorine dioxide (mg/liter;  $\bullet$ ) released into the atmosphere in the cabinet used to treat blueberries.

using sodium thiosulfate as a titrant (2). The detailed procedure and a description of  $\text{ClO}_2$  chemistry are published elsewhere (1).

Immediately following placement of the fruit samples on the bottom three shelves in the treatment cabinet, hot water (20 ml) in a plastic weigh dish was placed on the bottom shelf, whereas three sachets that contained the reactant chemicals were simultaneously placed on an elevated mesh platform placed on the top shelf to deliver maximum levels of relative humidity and desired concentrations of gaseous ClO<sub>2</sub>. The control samples were handled in an identical manner, except ClO<sub>2</sub> sachets were not placed in the cabinet. Closing and securing the door to which a rubber gasket was affixed sealed the cabinet.

Efficiency of recovery of *Salmonella*. Preliminary studies were conducted to determine the most efficient method to recover *Salmonella*, yeasts, and molds from blueberries, strawberries, and raspberries. The skin of fruits was inoculated with 100  $\mu$ l of *Salmonella* suspension to give 6.81 log CFU/g of blueberries and raspberries and 6.11 log CFU/g of strawberries. Two methods (washing and stomaching) were evaluated for their efficiency in removing *Salmonella* and naturally occurring yeasts and molds.

Strawberries on which *Salmonella* inoculum had dried for 2 h at  $22 \pm 2^{\circ}$ C were placed in a stomacher 400 bag (Seward Medical, London, England), and 100 ml of Dey-Engley (DE) broth (Difco, Becton Dickinson) was added. The strawberries were gently hand rubbed for 1 min to wash the external surface of each fruit. Samples of blueberries and raspberries inoculated with *Salmonella* on which 100 µl of *Salmonella* suspension had dried for 2 h at  $22 \pm 2^{\circ}$ C were placed in a stomacher 80 bag, and 40 ml of DE broth was added to each bag. Blueberries and raspberries were washed with DE broth by placing samples on a platform shaker (New Brunswick Scientific, Innova 2000, Brunswick, N.J.) and shaking at 150 rpm for 1 min.

Pummeling samples in a stomacher blender (Seward Medical) was also evaluated as a procedure to remove *Salmonella*, yeasts, and molds from berries. Samples were prepared as described for the wash method, except that, instead of washing berries, strawberries were pummeled at normal speed for 1 min in a stomacher 400 blender and blueberries and raspberries were pummeled for 1 min in a stomacher 80 blender.

Microbiological analyses. Undiluted DE wash broth and homogenates (0.25 ml in quadruplicate and 0.1 ml in duplicate), as



FIGURE 2. Atmospheric relative humidity (%;  $\blacksquare$ ,  $\blacktriangle$ ) and amount of chlorine dioxide (mg/liter;  $\bullet$ ) released into the atmosphere in the cabinet used to treat strawberries and raspberries.

well as samples (0.1 ml in duplicate) serially diluted in sterile 0.1% peptone, from berries examined in preliminary studies were surface plated on TSANP and xylose lysine desoxycholate agar (pH 7.4) supplemented with nalidixic acid (50 µg/ml) and 1 g/ liter of sodium pyruvate (XLDNP agar) to enumerate Salmonella. Dichloran rose bengal chloramphenicol (DRBC) agar (pH 5.6) (Difco, Becton Dickinson) was used to enumerate yeasts and molds. The TSANP and XLDNP agar plates were incubated at 37°C for 24 h before presumptive-positive Salmonella colonies were counted. Five to ten presumptive-positive colonies were randomly selected for confirmation using a Salmonella latex test (Oxoid, Basingstoke, England), lysine iron agar (Difco, Becton Dickinson), and triple sugar iron agar (Difco, Becton Dickinson). The DRBC agar plates were incubated at 25°C for 5 days before yeast and mold colonies were counted. Following removal of samples for plating on TSANP, XLDNP agar, and DRBC agar, the pH of DE wash broth and the berry and DE broth homogenates were measured.

Inoculated, untreated berry samples, as well as samples held for up to 120 min in air (control) or gaseous  $ClO_2$  in the cabinet, were analyzed for populations of *Salmonella*, yeasts, and molds according to the wash procedure described herein. Untreated and treated strawberries, blueberries, and red raspberries were analyzed.

Following removal of wash broth samples for plating on TSANP and DRBC agar, 40 ml of 2× lactose broth (Difco, Becton Dickinson) supplemented with nalidixic acid (50 µg/ml) and sodium pyruvate (1 g/liter) (LBNP) was added to each bag that contained DE wash broth and blueberries or raspberries that had been treated with gaseous ClO<sub>2</sub>; 100 ml of  $2 \times$  LBNP was added to each sample of strawberries in DE wash broth. Mixtures of fruit samples with DE broth and LBNP were incubated at 37°C for 24 h. If samples treated with ClO<sub>2</sub> did not yield one or more colonies of Salmonella on TSANP, the preenriched mixture was examined for the presence of Salmonella. A loop (approximately 10 µl) of each DE broth-berry-LBNP mixture was streaked on XLDNP agar and the plates were incubated at 37°C for 24 h before examining for the presence of presumptive Salmonella colonies. In addition, 0.1-ml samples of the preenriched mixture were inoculated into 10 ml of Rappaport-Vassiliadis enrichment broth (pH 5.1) (Difco, Becton Dickinson). The Rappaport-Vassiliadis broth was incubated at 42°C for 24 h. If samples of preenriched

		Population of Salmonella (log CFU/g) <sup>a</sup>									
	-	TSANP		XLDNP aga	XLDNP agar						
Fruit	method	Recovered $(SD)^b$	Reduction <sup>c</sup>	Recovered (SD) <sup>b</sup>	Reduction <sup>c</sup>	Recovered $(SD)^b$					
Strawberry	Inoculum	6.11		5.53							
-	Wash	A 5.23 (0.13)	0.88	А 4.72 (0.11)	0.51	A 4.18 (0.20)					
	Stomach	а 5.24 (0.10)	0.87	в 4.22 (0.30)	1.01	в 3.79 (0.02)					
Blueberry	Inoculum	6.81		6.23							
	Wash	A 6.49 (0.25)	0.32	a 5.36 (0.20)	0.87	A 2.00 (2.01)					
	Stomach	в 5.49 (0.14)	1.32	в 4.31 (0.14)	1.92	a 1.52 (1.73)					
Raspberry	Inoculum	6.81		6.23							
	Wash	A 6.09 (0.20)	0.72	a 5.35 (0.13)	0.88	A 4.02 (0.19)					
	Stomach	A 5.97 (0.09)	0.84	a 5.07 (0.15)	1.16	A 4.28 (0.12)					

TABLE 1. Recovery of Salmonella, yeasts, and molds from strawberries, blueberries, and raspberries as affected by method of retrieval of cells from fruits

<sup>a</sup> Populations of *Salmonella* recovered on TSANP and XLDNP agar and yeasts and molds on DRBC agar after washing or stomaching strawberries, blueberries, and raspberries. The population of *Salmonella* (log CFU/g of fruit) in inoculum was calculated based on the number of CFUs recovered by plating diluted samples of inoculum on TSANP and XLDNP agar.

<sup>b</sup> Within each fruit and recovering medium, mean values not preceded by the same letter are significantly different ( $\alpha = 0.05$ ).

<sup>c</sup> Reduction (log CFU/g) compared with the number in the inoculum recovered on TSANP or XLDNP agar.

mixture did not yield presumptive-positive *Salmonella* colonies on the XLDNP agar plates, a loopful of 24-h Rappaport-Vassiliadis culture was streaked on XLDNP agar. Plates were incubated at 37°C for 24 h before examining for presumptive *Salmonella* colonies, followed by confirmation.

Sensory evaluation. An untrained panel of 31 individuals from the Center for Food Safety and Department of Food Science and Technology subjectively evaluated treated (4.1 mg/liter of ClO<sub>2</sub>) and untreated, uninoculated blueberries, strawberries, and raspberries. Panelists were familiarized with sensory qualities of berries through discussions and a 20-min examination of visual and aroma characteristics of fresh, untreated blueberries, strawberries, and raspberries. Both treated and control berries were placed in covered plastic bins (50.8 cm long by 34.3 cm wide by 12.7 cm high) and stored for 0, 3, 7, and 10 days at 8°C before evaluating for sensory quality. Samples that consisted of 20 g of blueberries, 100 g of strawberries, or 20 g of raspberries in white plastic weigh boats were placed on a white surface and assigned random three-digit codes. These samples were presented to the panel in random order. Panelists were asked to carefully examine the appearance, color, aroma, and overall quality of the berries. Sensory attributes were rated by assigning scores of 1 to 9 on a 9-point hedonic scale, with 1 indicating dislike extremely, 5 indicating neither like nor dislike, and 9 indicating like extremely. All evaluations were conducted within 90 min after treating the samples with ClO<sub>2</sub> gas (day 0) or within 1 h of removing the samples from storage at 8°C.

Statistical analyses. Preliminary experiments consisted of three samples of each fruit subjected to each recovery method. All other experiments were replicated at least three times, and each replicate experiment consisted of three samples exposed to the same treatment conditions. Undiluted washes or homogenates were plated in quadruplicate (0.25-ml samples) or duplicate (0.1ml samples), and serially diluted samples were plated in duplicate (0.1-ml samples). Mean values were analyzed to determine significant differences ( $\alpha = 0.05$ ) in populations of *Salmonella* or yeasts and molds on fruits subjected to various recovery methods and ClO<sub>2</sub> treatment concentrations. Mean values were also analyzed to determine statistically significant differences ( $\alpha = 0.05$ ) in sensory attributes of berries as affected by treatment. Data were subjected to SAS statistical software analysis (SAS Institute Inc., Cary, N.C.) for analysis of variance and Duncan's multiple range tests.

#### **RESULTS AND DISCUSSION**

Dip, spray, and spot application are among the options for inoculating the surface of fruits and vegetables with pathogens for the purpose of conducting sanitizer efficacy studies (7). Spot inoculation was chosen because it mimics contamination that might occur in the field, during harvesting, in packinghouses, or in foodservice or home settings as a result of contact of fruits with animals, fecal material, or contaminated processing equipment or handlers. A known number of cells can be applied to produce using spot inoculation, enabling reductions in the number of viable cells caused by sanitization treatment to be more accurately calculated (31).

Comparison of recovery methods. Preliminary studies that compared washing and stomaching as methods to recover Salmonella revealed that the number of Salmonella recovered on TSANP from stomached blueberries was significantly lower ( $\alpha = 0.05$ ) than the number recovered by washing the berries (Table 1). This observation is contrary to that of others (30) in which surface rinsing recovered slightly lower populations of Salmonella on strawberries than those recovered by stomaching. Han and Linton (20), on the other hand, reported that acids in strawberry juice appeared to injure or inactivate E. coli O157:H7 and L. monocytogenes. Stomaching strawberries, when compared with washing to remove E. coli O157:H7 and L. monocytogenes, has been reported to reduce the recovery of the two pathogens (21). This was attributed to injury of cells exposed to juice released from the strawberries during the stomaching process. Populations recovered from stomached blueberries were significantly lower ( $\alpha = 0.05$ ) compared with populations recovered from washed blueberries when samples were plated on XLDNP agar. The number of *Salmonella* recovered from stomached strawberries and raspberries was significantly lower ( $\alpha = 0.05$ ) compared with populations recovered from washed berries when samples were plated on XLDNP agar.

Populations of yeasts and molds recovered from stomached and washed berries are also given in Table 1. Stomached strawberries but not blueberries and raspberries showed significantly lower ( $\alpha = 0.05$ ) populations of yeasts and molds compared with washed berries.

Stomaching results in the release of acidic juice from berry tissues, which decreased the pH of the DE broth (7.60) to 4.05, 4.09, and 4.04 in blueberry, strawberry, and raspberry homogenates, respectively, and may have caused injury or death of some of the Salmonella. Although some salmonellae can survive in acidic environments (pH > 4.0), they cannot survive in foods with lower pH or at higher pH in foods that contain certain types of acids (11). Since the pH of strawberries, blueberries, and raspberries ranges from 2.9 to 3.5 (42), release of tissue fluids caused by stomaching may have caused death and sublethal injury of Salmonella. Debilitation of cells may further contribute to reduced numbers of Salmonella recovered from homogenized fruits on XLDNP agar. Washing, rather than homogenizing, was used as the processing method to remove Salmonella, yeasts, and molds from the surface of berries in subsequent studies that evaluated  $ClO_2$  as a sanitizer.

A nutrient-rich medium (TSANP) selective for nalidixic acid-adapted cells was used to enumerate Salmonella on inoculated fruits. Preliminary studies with uninoculated samples showed that most background microflora did not grow on TSANP or XLDNP agar. The performance of TSANP was equal to or better than XLDNP agar for supporting colony development by Salmonella (Table 1). To maximize the recovery of ClO2-stressed Salmonella, direct plating on TSANP was preferred over plating on XLDNP agar, which contains ingredients that may impose secondary stresses. Han et al. (24) observed that surface plating on conventional selective media resulted in poor recovery of E. coli O157:H7 and L. monocytogenes on green peppers treated with ClO<sub>2</sub>. Although higher numbers of both pathogens were recovered from peppers subjected to resuscitation procedures, counts were still lower than those obtained on nonselective media.

**Relative humidity.** A high relative humidity (75 to 90%) was maintained in the chamber atmosphere (Figs. 1 and 2) to enhance the lethality of  $ClO_2$  to *Salmonella*, yeasts, and molds. The lethality of gaseous  $ClO_2$  to *E. coli* O157:H7 on peppers is known to increase as the atmospheric relative humidity is increased (18). Treatment of juice storage tanks with 8 mg/liter of  $ClO_2$  has been shown to also be more effective in killing spoilage microorganisms as the relative humidity is increased from 56 to 94% (19).

**Treatment of blueberries.** Blueberries were inoculated on the skin, calyx, or stem scar tissue with concentrations of 4.1, 6.2, and 8.0 mg/liter within 30, 60, and 120 min, respectively, at 76 to 90% relative humidity and  $23 \pm$ 

1°C. Log reductions of Salmonella resulting from these treatments are given in Table 2. Compared with blueberries not treated with ClO<sub>2</sub> (control), all treatments with gaseous  $ClO_2$  caused significant reductions ( $\alpha = 0.05$ ) in the number of viable cells, regardless of the site on which inoculum was applied. The highest log reductions on blueberries inoculated at all three locations resulted from treatment with 8.0 mg/liter of  $ClO_2$  (2.4 to 3.7 log CFU/g), although reductions achieved using 4.1, 6.2, or 8.0 mg/liter of ClO<sub>2</sub> were similar. There was no significant difference between the sanitizing effect of gaseous  $ClO_2$  gas on the population of Salmonella inoculated onto the calyx and stem scar tissue. However, there was a significantly higher reduction ( $\alpha$ = 0.05) on blueberries inoculated on the skin and treated with gaseous  $ClO_2$  at concentrations of 4.1 or 6.2 mg/liter compared with reductions on blueberries on which the inoculum had been applied to the stem scar tissue and treated with respective concentrations of ClO<sub>2</sub>. This is attributed to a larger percentage of cells on the skin being exposed to ClO2 and therefore more vulnerable to its lethality compared with cells protected by stem scar tissues. Results are in agreement with observations on the higher reductions in populations of L. monocytogenes on the skin (5.5-log reduction) versus stem cavity (3.6-log reduction) or calyx (3.2-log reduction) of apples treated with gaseous  $ClO_2$  at 4.0 mg/liter for 10 min at 21°C and 90% relative humidity (15). Reductions were 1 to 3 log higher than those achieved in our study using 4.1 mg/liter of ClO<sub>2</sub> to treat blueberries. Differences in sensitivity of various foodborne bacterial pathogens and differences in surface structures and morphology of apples and blueberries may result in differences in the level of protection of cells against exposure to ClO<sub>2</sub>, which in turn may have affected its biocidal efficacy in these studies. Different systems were used to produce gaseous ClO<sub>2</sub> in the two studies. The sachets used in our study released 4.1 mg/liter within 30 min, whereas the ClO2 gas treatment system used by Du et al. (15) released 4.0 mg/ liter within seconds, enabling a shorter exposure time (10 min) to achieve reductions in pathogen populations greater than those we observed after treatment for 30 min.

The yeast and mold populations on blueberries were significantly reduced ( $\alpha = 0.05$ ), compared with those detected in control samples by treating with gaseous ClO<sub>2</sub> at 4.1 mg/liter, regardless of the site of inoculation with *Salmonella* (Table 2). No significant differences occurred ( $\alpha = 0.05$ ) in log reductions in yeast and mold populations on blueberries inoculated with *Salmonella* at a specific site and treated with 4.1, 6.2, or 8.0 mg/liter of ClO<sub>2</sub>. In addition, no significant differences occurred in log reductions in yeast and mold populations on blueberries inoculated sites and treated with *Salmonella* at three different sites and treated with the same concentration of ClO<sub>2</sub>. The natural distribution and survival of yeasts and molds on the blueberries were apparently unaffected by handling or the procedure used to inoculate with *Salmonella*.

**Treatment of strawberries.** Strawberries inoculated with *Salmonella* on the skin or stem scar area were treated with gaseous  $ClO_2$  at release concentrations of 4.1, 6.2, and

FABLE 2.	Recovery of	<sup>c</sup> Salmonella,	veasts,	and molds	from	blueberries	treated with	gaseous (	$ClO_{2}$	,
	2.1	,	~ /		./			()		

		Treatment time (min)	Amount of	Population (log CFU/g) <sup>b</sup>			
Microorganisms	Inoculation site <sup>a</sup>		ClO <sub>2</sub> released — (mg/liter)	Recovered (SD) <sup>c</sup>	Reduction <sup>d</sup>	Enrichment <sup>e</sup>	
Salmonella	Skin	0	0	a 4.94 (1.80) x			
		30	4.1	в 2.62 (0.53) х	2.95	2/2	
		60	6.2	в 1.38 (1.31) у	3.56	4/5	
		120	8.0	в 1.27 (1.10) у	3.67	3/5	
	Calyx	0	0	a 5.13 (1.87) x			
		30	4.1	в 2.93 (0.00) х	2.20		
		60	6.2	в 3.25 (0.59) х	1.88		
		120	8.0	в 2.69 (0.52) х	2.44	2/3	
	Stem scar	0	0	a 5.86 (2.09) x			
		30	4.1	вс 3.43 (0.38) х	2.43	1/1	
		60	6.2	в 3.50 (0.10) х	2.36		
		120	8.0	с 2.62 (0.52) х	3.24	1/2	
Yeasts and molds	Skin	0	0	a 3.41 (1.31) x			
		30	4.1	в 0.71 (0.69) х	2.70		
		60	6.2	в 0.63 (0.74) х	2.78		
		120	8.0	в 1.09 (1.10) х	2.32		
	Calyx	0	0	a 3.40 (1.21) x			
		30	4.1	в 1.99 (1.62) х	1.41		
		60	6.2	в 1.14 (0.13) х	2.26		
		120	8.0	в 0.84 (0.13) х	2.51		
	Stem scar	0	0	a 3.39 (1.32) x			
		30	4.1	в 0.87 (0.30) х	2.52		
		60	6.2	в 1.01 (0.37) х	2.38		
		120	8.0	в 1.33 (0.20) х	2.06		

<sup>a</sup> Salmonella was inoculated on the skin, calyx, or stem scar of blueberries.

<sup>b</sup> Populations of Salmonella recovered on TSANP and yeasts or molds recovered on DRBC agar after treatment of blueberries for 0, 30, 60, and 120 min at 23°C. The population of Salmonella inoculated onto blueberries was 6.51 (±0.11) log CFU/g. The detection limit was 1 CFU/2 ml of DE wash (1 CFU/g of blueberries).

<sup>c</sup> Within the same microorganism and inoculation site, mean values not preceded by the same letter are significantly different ( $\alpha = 0.05$ ). Within the same microorganism and ClO<sub>2</sub> concentration, mean values not followed by the same letter are significantly different ( $\alpha = 0.05$ ).

<sup>d</sup> Within the same microorganism and inoculation site, reduction (log CFU/g) compared with the population recovered from blueberries receiving no ClO<sub>2</sub> treatment (0 mg/liter).

<sup>e</sup> Number of samples positive for Salmonella/number of samples analyzed of treated, washed blueberries as detected by enrichment. Samples on which Salmonella was recovered by direct plating were not analyzed by enrichment.

8.0 mg/liter within 30, 60, and 120 min, respectively, at 85 to 88% relative humidity and 23  $\pm$  1°C. Populations of the pathogen recovered are given in Table 3. Treatment with 4.1 mg/liter of ClO<sub>2</sub> caused significant reductions ( $\alpha$  = 0.05) in populations of Salmonella of 2.22 and 2.32 log CFU/g of strawberries inoculated on the stem scar and skin, respectively. Higher reductions were caused by treatment with 6.2 and 8.0 mg/liter of ClO<sub>2</sub> compared with treatment with 4.1 mg/liter. Unlike blueberries, the effectiveness of a given concentration of ClO2 in killing Salmonella on strawberries was unaffected by the site of inoculation. This may be in part due to similarities in porosity of skin and stem scar surfaces and is in contrast to the protective effect afforded by the stem scar tissues in blueberries. Han et al. (25) reported that gaseous  $ClO_2$  was effective in killing E. coli O157:H7 and L. monocytogenes on strawberries. Rogers et al. (36) examined the effectiveness of aqueous ClO<sub>2</sub> in killing E. coli O157:H7 and L. monocytogenes on raw produce. Treatment of strawberries with 3 or 5 mg/liter of  $ClO_2$  for 20 to 30 s caused approximately a 5-log reduction in population. Initial populations of *E. coli* O157:H7 and *L. monocytogenes* of 6.1 and 5.8 log CFU/g, respectively, were reduced to less than 1 log CFU/g within a 5-min treatment with 3 mg/liter of ClO<sub>2</sub>. These larger reductions, compared with those observed for *Salmonella* in our study, may have resulted from differences in sensitivity of pathogens to ClO<sub>2</sub> and to other experimental parameters but are more likely a reflection of different methods of inoculation and retrieval of viable cells from treated strawberries.

The yeast and mold populations on strawberries were significantly reduced when treated with gaseous  $ClO_2$  (Table 3). Populations recovered from strawberries treated with 4.1, 6.2, and 8.0 mg/liter of gaseous  $ClO_2$  were not significantly different, but treatment with 8.0 mg/liter caused substantially higher reductions (4.07 and 4.16 log CFU/g on the stem scar and skin, respectively) compared with treatment with 6.2 mg/liter (2.90 and 1.53 log CFU/g, respectively). The greater effectiveness of gaseous  $ClO_2$  at a concentration of 8.0 mg/liter in killing yeasts and molds on strawberries compared with blueberries may have been

		Treatment time (min)	Amount of	Population (log CFU/g) <sup><math>a</math></sup>			
Microorganisms	Inoculation site		ClO <sub>2</sub> released — (mg/liter)	Recovered (SD) <sup>b</sup>	Reduction <sup>c</sup>	Enrichment <sup>d</sup>	
Salmonella	Skin	0	0	a 4.43 (0.86) x			
		30	4.1	в 2.11 (0.76) х	2.32		
		60	6.2	вс 1.10 (0.50) х	3.33	0/1	
		120	8.0	c 0.67 (0.60) x	3.76	0/6	
	Stem scar	0	0	a 4.67 (0.60) x			
		30	4.1	в 2.45 (1.04) х	2.22	0/1	
		60	6.2	в 1.87 (1.18) х	2.80	1/4	
		120	8.0	c 0.26 (0.28) x	4.41	1/4	
Yeasts and molds	Skin	0	0	a 4.87 (0.63) x			
		30	4.1	в 3.45 (1.65) х	1.42		
		60	6.2	в 3.34 (0.76) х	1.53		
		120	8.0	с 0.71 (0.66) х	4.16		
	Stem scar	0	0	a 4.86 (0.58) x			
		30	4.1	в 2.58 (0.25) х	2.28		
		60	6.2	в 1.96 (0.44) х	2.90		
		120	8.0	с 0.79 (0.16) х	4.07		

TABLE 3. Recovery of Salmonella, yeasts, and molds from strawberries treated with gaseous ClO<sub>2</sub>

<sup>*a*</sup> Populations of *Salmonella* recovered on TSANP and yeasts or molds recovered on DRBC agar after treatment of strawberries for 0, 30, 60, and 120 min at 23°C. The population of *Salmonella* inoculated onto strawberries was 6.05 ( $\pm$ 0.28) log CFU/g. The detection limit was 1 CFU/2 ml of DE wash (1 CFU/g of strawberries).

<sup>b</sup> Within the same microorganism and inoculation site, mean values not preceded by the same letter are significantly different ( $\alpha = 0.05$ ). Within the same microorganism and ClO<sub>2</sub> concentration, mean values not followed by the same letter are significantly different ( $\alpha = 0.05$ ).

<sup>c</sup> Within the same microorganism and inoculation site, reduction (log CFU/g) compared with the population recovered from strawberries receiving no ClO<sub>2</sub> treatment (0 mg/liter).

<sup>d</sup> Number of samples positive for *Salmonella*/number of samples analyzed of treated, washed strawberries as detected by enrichment. Samples on which *Salmonella* was recovered by direct plating were not analyzed by enrichment.

caused by the slightly higher relative humidity achieved and maintained in the cabinet during treatment of strawberries (Fig. 2) versus blueberries (Fig. 1), attesting to the synergistic role high relative humidity plays in enhancing the lethality of gaseous ClO<sub>2</sub>. On the other hand, strawberries had a higher initial population of yeasts and molds (4.86 to 4.87 log CFU/g) compared with populations on blueberries (3.39 to 3.41 log CFU/g). Different genera or species of yeasts and molds on the three types of berries and the number of mycelia, spores, and conidia would also be expected to vary in sensitivity to ClO<sub>2</sub>, and this would be reflected in differences in reductions in counts that resulted from treatment.

**Treatment of raspberries.** Raspberries inoculated with *Salmonella* only at one location, the skin, were treated with gaseous ClO<sub>2</sub> at release concentrations of 4.1, 6.2, and 8.0 mg/liter within 30, 60, and 120 min, respectively, at 75 to 83% relative humidity and  $23 \pm 1^{\circ}$ C. Reductions in populations of *Salmonella* are given in Table 4. Treatment with gaseous ClO<sub>2</sub> significantly ( $\alpha = 0.05$ ) reduced the number of *Salmonella*. No significant difference occurred between the log reductions caused by treating raspberries with the three concentrations of ClO<sub>2</sub>. The lower reductions in populations of *Salmonella* on raspberries (0.52 to 1.54 log CFU/g) compared with reductions on blueberries and strawberries treated with the same concentrations of ClO<sub>2</sub> are attributed in part to the lower relative humidity during treat-

ment of raspberries (Fig. 2) compared with blueberries and strawberries (Figs. 1 and 2, respectively) with ClO<sub>2</sub>. Failure to achieve a higher relative humidity may have been influenced by the high respiration rate (114 to 245 mg CO<sub>2</sub>/kg/ h at 20°C) of raspberries compared with the respiration rate of strawberries (102 to 196 mg CO<sub>2</sub>/kg/h at 20°C) and blueberries (52 to 87 mg CO<sub>2</sub>/kg/h at 20°C) (37). The evolution of higher amounts of CO2 associated with the higher respiration rate of raspberries may protect the surface from contact with gaseous ClO<sub>2</sub>, thereby potentially reducing the lethality of ClO<sub>2</sub> to Salmonella. Reduced access of ClO<sub>2</sub> to areas between the drupelets of raspberries where part of the inoculum may have lodged would also protect Salmonella from exposure to ClO2. Tissue juice released from broken trichomes may provide a site for harborage for the pathogen. Some or all of these factors may have affected the efficacy of gaseous ClO<sub>2</sub> in killing Salmonella on raspberries to a greater extent than on blueberries or strawberries. Treatment of uninjured green pepper surfaces with gaseous ClO<sub>2</sub> at a concentration of 0.60 mg/liter for 30 min at 20°C under 90 to 95% relative humidity has been reported to result in a reduction in population of E. coli O157:H7 of 7.27 log CFU/5 g (22). Treatment of injured green pepper surfaces (crevices created uniformly by cutting of sterile blade) with 1.2 mg/liter of ClO<sub>2</sub> resulted in a 6.45-log CFU/ 5 g reduction. Their study also demonstrated, through counts of CFUs and confocal laser scanning microscopy,

TABLE 4.	Recovery of	Salmonella,	yeasts, an	d molds from	the skin	surface o	f raspberries	treated with	gaseous (	$ClO_2$	
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		Amount of	Population (log CFU/g) <sup>a</sup>					
Microorganism	Treatment time (min)	ClO <sub>2</sub> released (mg/liter)	Recovered $(SD)^b$	Reduction <sup>c</sup>	Enrichment <sup>d</sup>			
Salmonella	0	0	a 4.83 (1.01)					
	30	4.1	авс 4.31 (1.19)	0.52				
	60	6.2	вс 3.77 (2.19)	1.06	1/3			
	120	8.0	с 3.29 (0.60)	1.54	0/3			
Yeasts and molds	0	0	a 4.04 (1.15)					
	30	4.1	в 1.02 (0.77)	3.02				
	60	6.2	в 0.86 (0.27)	3.18				
	120	8.0	в 1.48 (0.80)	2.56				

<sup>a</sup> Populations of Salmonella recovered on TSANP and yeasts and molds recovered on DRBC agar after treatment of raspberries for 0, 30, 60, and 120 min at 23°C. The population of Salmonella inoculated onto raspberries was 6.78 (±0.28) log CFU/g. The detection limit was 1 CFU/2 ml of DE wash (1 CFU/g of raspberries).

<sup>b</sup> Within microorganism, mean values not preceded by the same letter are significantly different ( $\alpha = 0.05$ ).

<sup>c</sup> Within microorganism, reduction (log CFU/g) compared with the population recovered from raspberries receiving no  $CIO_2$  treatment (0 mg/liter).

<sup>d</sup> Number of samples positive for *Salmonella*/number of samples analyzed of treated, washed raspberries as detected by enrichment. Samples on which *Salmonella* was recovered by direct plating were not analyzed by enrichment.

that *E. coli* O157:H7 preferentially attached to injured surfaces on peppers, which protected it from contact with ClO<sub>2</sub>, resulting in significantly lower reductions in viable cells. Another study showed that *E. coli* O157:H7 preferentially attached to coarse and porous intact surfaces and injured surfaces of peppers (*26*). Similar protective phenomena may have been exhibited by raspberries and, to a lesser extent, by blueberries and strawberries examined in our study.

Populations of yeasts and molds on raspberries treated with gaseous  $ClO_2$  were significantly reduced compared with populations on control raspberries (Table 4). However, as with reductions in populations of *Salmonella*, differences in populations of yeasts and molds recovered from raspberries treated with 4.1, 6.2, or 8.0 mg/liter were not significantly different ( $\alpha = 0.05$ ).

Sensory evaluation of berries. The efficacy of 4.1 mg/ liter of ClO<sub>2</sub> in removing or killing pathogens met recommendations by an Environmental Protection Agency Scientific Advisory Panel, wherein sanitizers tested against at least five strains of outbreak-related Salmonella would result in a reasonable performance standard of a 2-log reduction in population (17). Berries were therefore treated with 4.1 mg/liter of  $ClO_2$  to determine if sensory attributes are maintained while simultaneously achieving approximate 2log CFU/g reductions. Temperatures during produce transport and in retail produce display areas can range from 9 to 12°C, whereas temperatures in home refrigerators may reach 10°C or higher (10, 27, 34, 43). According to Ryall and Pentzer (38), typical storage lives of blueberries, strawberries, and raspberries stored at 0°C and 90 to 95% relative humidity are approximately 2 weeks, 5 to 7 days, and 3 days, respectively. Storage times of up to 10 days at 8°C with evaluation sessions on days 0, 3, 7, and 10 were selected to determine if changes in sensory attributes of berries result from treatment with ClO<sub>2</sub>.

Sensory quality of control and treated (4.1 mg/liter of ClO<sub>2</sub>) blueberries was not significantly different ( $\alpha = 0.05$ ) on days 3, 7, or 10 of storage (Table 5). Untreated berries were rated between 5.4 and 6.9 for appearance, 5.8 and 7.0 for color, 5.2 and 5.4 for aroma, and 5.4 and 6.6 for overall quality during the 10-day storage period; for treated blueberries, ratings were 5.8 to 7.1, 6.0 to 7.2, 5.2 to 5.4, and 5.7 to 6.8, respectively. However, ratings for appearance, color, overall quality, and, to a lesser extent, aroma of control and treated blueberries decreased from initial ratings of "like slightly" and "like moderately" (6 to 7) to "neither like nor dislike" and "like slightly" (5 to 6) during the 10day storage period. The relative humidity of the atmosphere that surrounded untreated blueberries declined from 58 initially to 38% during storage. This may have been caused by removal of samples from the storage bin at 3 and 7 days, thereby removing biomass that would otherwise contribute moisture to the air inside the bins. The reduction in relative humidity may have contributed to a loss in sensory quality of blueberries as storage time increased.

The appearance, color, aroma, and overall ratings were not significantly different ( $\alpha = 0.05$ ) for untreated and treated (4.1 mg/liter of ClO<sub>2</sub>) strawberries after storage for 3, 7, or 10 days (Table 5). Bleached spots on treated strawberries observed on day 0 (within 90 min after treatment) were not as evident on days 3, 7, and 10. Storage time affected sensory attributes of treated and control strawberries, as shown by significant decreases ( $\alpha = 0.05$ ) in ratings on each successive day of analysis. The shelf life of strawberries, in general, is much shorter than blueberries. The growth of Botrytis cinerea and other molds on untreated strawberries on days 7 and 10, as well as on treated strawberries on day 10, indicates that treatment with  $ClO_2$  may have retarded but did not prevent deterioration of the fruit. A higher water content of strawberries (approximately 90%) compared with that of raspberries (approximately

1173

		Treatment with ClO <sub>2</sub> - (mg/liter)	Ratings for sensory attributes <sup>a</sup> :						
Fruit	Storage time (days)		Appearance	Color	Aroma	Overall quality			
Blueberry	0	0	a 6.9 x	а 7.0 х	а 5.4 х	a 6.6 x			
5		4.1	а 7.1 х	а 7.2 х	а 5.4 х	a 6.8 x			
	3	0	в 6.3 х	в 6.4 х	ab 5.3 x	в 6.0 х			
		4.1	в 6.3 х	в 6.5 х	a 5.3 x	в 6.1 х			
	7	0	с 5.6 х	с 6.0 х	ab 5.3 x	с 5.5 х			
		4.1	с 5.8 х	вс 6.1 х	а 5.2 х	в 5.7 х			
	10	0	с 5.4 х	с 5.8 х	в 5.2 х	с 5.4 х			
		4.1	с 5.8 х	с 6.0 х	a 5.3 x	в 5.7 х			
Strawberry	0	0	а 7.2 х	а 7.3 х	а 7.5 х	а 7.1 х			
		4.1	а 6.5 у	A 6.6 Y	a 6.9 y	а 6.4 у			
	3	0	в 5.0 х	в 5.7 х	в 6.9 х	в 5.6 х			
		4.1	в 5.4 х	в 5.6 х	a 6.6 x	в 5.6 х			
	7	0	с 4.2 х	с 4.7 х	в 6.6 х	с 4.5 х			
		4.1	с 4.6 х	с 5.0 х	a 6.5 x	с 4.8 х			
	10	0	d 2.1 x	d 2.3 x	с 3.8 х	d 2.2 x			
		4.1	d 3.2 x	d 3.6 x	в 5.8 х	d 3.6 x			
Raspberry	0	0	a 6.9 x	a 6.9 x	a 6.0 x	а 6.7 х			
		4.1	а 6.5 ч	а б.б х	a 6.2 x	а 6.4 у			
	3	0	в 5.9 х	в 5.9 х	в 5.5 ч	в 5.6 х			
		4.1	в 6.0 х	в 6.1 х	a 5.9 x	в 5.9 х			
	7	0	с 4.9 х	с 4.8 х	в 5.4 х	с 4.7 х			
		4.1	с 4.6 х	с 4.5 х	в 5.4 х	с 4.5 х			
	10	0	с 4.5 х	с 4.5 х	в 5.6 х	с 4.5 х			
		4.1	с 4.2 х	с 4.2 х	с 5.0 х	с 4.1 х			

TABLE 5. Mean hedonic ratings for sensory attributes of uninoculated blueberries, strawberries, and raspberries exposed to 4.1 mg/ liter of  $ClO_2$  and held at 8°C for up to 10 days

<sup>*a*</sup> Ratings were assigned by panelists using a 9-point hedonic scale with 1 indicating dislike extremely, 5 indicating neither like nor dislike, and 9 indicating like extremely. Within the same fruit, treatment, and attribute, mean values not preceded by the same letter are significantly different ( $\alpha = 0.05$ ). Within the same fruit, storage time, and attribute, mean values not followed by the same letter are significantly different ( $\alpha = 0.05$ ).

84%) and blueberries (approximately 82%) (*38*) may have enhanced the growth of molds. Overall, sensory attribute ratings for untreated strawberries declined from "like moderately" to "dislike very much" (approximately 7 to 2), whereas ratings for treated strawberries declined from "like slightly" to "dislike moderately" (approximately 6 to 3) during the 10-day storage period, indicating that although treated strawberries initially had significantly lower ratings than untreated strawberries, their shelf life is slightly longer.

Sensory attributes of untreated and treated (4.1 mg/ liter) raspberries stored for 3, 7, or 10 days were not significantly different ( $\alpha = 0.05$ ) (Table 5). Ratings generally decreased significantly between 0 and 3 days and again between 7 and 10 days, regardless of treatment. The overall quality of untreated and treated raspberries on day 0 was rated as "like slightly" or "like moderately" (6 to 7) by the panelists. On day 3, untreated and treated raspberries were rated as "neither like nor dislike" or "like slightly" (5 to 6), whereas on days 7 and 10 ratings were reduced to "dislike slightly" or "neither like nor dislike" (4 to 5). The shelf life of raspberries is limited (38), owing in part to its high respiration rate. The decline in sensory quality of raspberries within 10 days was therefore expected. The panelists did not distinguish differences between untreated and treated raspberries, except for appearance and overall quality on day 0. This suggests that treatment with 4.1 mg/liter of  $ClO_2$  gas does not adversely affect the shelf life of raspberries. The relative humidity in the bin that contained untreated raspberries was higher (53 to 70%) compared with bins that contained treated raspberries (45 to 66%) but increased as storage time progressed, a trend similar to that noted for blueberries and strawberries.

In summary, gaseous ClO<sub>2</sub> shows promise as a sanitizer for small fruits. Significant reductions in Salmonella populations of 1.9 to 3.7, 2.2 to 4.4, and 0.5 to 1.5 log CFU/g of blueberries, strawberries, and raspberries, respectively, were achieved by treatment with 4.1 to 8.0 mg/liter of ClO<sub>2</sub> at elevated relative humidity. Treatment also reduced the yeast and mold populations by 1.4 to 2.8, 1.4 to 4.2, and 2.6 to 3.1 log CFU/g of blueberries, strawberries, and raspberries, respectively. Sensory ratings for appearance, color, and aroma of blueberries treated with gaseous 4.1 mg/liter of  $ClO_2$  were similar to ratings for untreated blueberries. Attributes of treated strawberries and raspberries were significantly lower than those of respective untreated fruits on day 0 only. Overall, approximately 2-log reductions of Salmonella populations were achieved by treatment of blueberries and strawberries with 4.1 mg/liter of ClO<sub>2</sub> without compromising sensory quality. Additional research is needed to determine the effectiveness of application of gaseous  $ClO_2$  on a commercial scale.

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