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Evaluation of Gaseous Chlorine Dioxide as a Sanitizer for Killing *Salmonella, Escherichia coli* O157:H7, *Listeria monocytogenes,* and Yeasts and Molds on Fresh and Fresh-Cut Produce

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ABSTRACT

Gaseous chlorine dioxide (ClO_2) was evaluated for effectiveness in killing *Salmonella, Escherichia coli* O157:H7, and *Listeria monocytogenes* on fresh-cut lettuce, cabbage, and carrot and *Salmonella,* yeasts, and molds on apples, peaches, tomatoes, and onions. Inoculum (100 μl, ca. 6.8 log CFU) containing five serotypes of *Salmonella enterica,* five strains of *E. coli* O157:H7, or five strains of *L. monocytogenes* was deposited on the skin and cut surfaces of fresh-cut vegetables, dried for 30 min at 22°C, held for 20 h at 4°C, and then incubated for 30 min at 22°C before treatment. The skin surfaces of apples, peaches, tomatoes, and onions were inoculated with 100 μl of a cell suspension (ca. 8.0 log CFU) containing five serotypes of *Salmonella,* and inoculated produce was allowed to dry for 20 to 22 h at 22°C before treatment. Treatment with ClO_2 at 4.1 mg/liter significantly ($\alpha = 0.05$) reduced the population of foodborne pathogens on all produce. Reductions resulting from this treatment were 3.13 to 4.42 log CFU/g for fresh-cut cabbage, 5.15 to 5.88 log CFU/g for fresh-cut carrots, 1.53 to 1.58 log CFU/g for fresh-cut lettuce, 4.21 log CFU per apple, 4.33 log CFU per tomato, 1.94 log CFU per onion, and 3.23 log CFU per peach. The highest reductions in yeast and mold populations resulting from the same treatment were 1.68 log CFU per apple and 2.65 log CFU per peach. Populations of yeasts and molds on tomatoes and onions were not significantly reduced by treatment with 4.1 mg/liter ClO_2. Substantial reductions in populations of pathogens on apples, tomatoes, and onions but not peaches or fresh-cut cabbage, carrot, and lettuce were achieved by treatment with gaseous ClO_2 without markedly adverse effects on sensory qualities.

The frequency of outbreaks of enteric infections associated with consumption of raw fruits and vegetables has been increasing. Possible causes for this include increased importation to the United States of produce from countries where hygienic practices used to grow and handle produce may be compromised, changes in production, handling, and processing practices, contamination in food service and home preparation settings, and increased consumption of fresh and minimally processed produce *(5, 17, 30, 32, 33, 41).* Treatment of fruits and vegetables with sanitizers often results in reductions in populations of pathogens not exceeding 2 to 3 log CFU/g *(4)* and cannot be relied upon to eliminate safety risks. The lack of effectiveness of sanitizers for killing high numbers of pathogens on produce can be attributed in part to difficulties in delivering aqueous chemical sanitizers to surface or subsurface areas where pathogens may be lodged *(9).* Treatment with aqueous chemical solutions can result in residual moisture on the surface of fruits and vegetables, which can promote the growth of yeasts and molds, thus reducing fresh-market shelf life. Growth of molds can in turn increase the pH of produce tissues and enhance the growth of infectious and toxigenic foodborne pathogens *(13, 43),* thereby increasing safety risks.

Gaseous acetic acid *(10, 37, 39, 44),* hydrogen peroxide *(40),* and chlorine dioxide (ClO_2) *(14, 15, 19–25, 29, 42)* have been evaluated as alternatives to aqueous chemicals for sanitizing fresh and minimally processed fresh-cut fruits and vegetables. Application of 4.0 mg/liter of ClO_2 gas to apples, for example, resulted in a 5.5-log reduction in the number of *Listeria monocytogenes (14).* The pathogen was reduced by 7.4 log CFU/5 g of green peppers by treating with 3 mg/liter ClO_2 gas *(22).* For surface-injured green peppers, treatment with 1.2 mg/liter ClO_2 gas resulted in a 6.4 log CFU reduction in *Escherichia coli* O157:H7 per spotted site compared with a reduction of 1.5 to 1.7 log CFU per spotted site achieved by washing injured surfaces with water *(25).*

Gaseous ClO_2 also can reducing yeast and mold populations in food processing plants and on fruits and vegetables. In a companion study *(42),* we reported that treatment of blueberries, strawberries, and raspberries with 4.1 mg/liter ClO_2 caused significant reductions in populations of *Salmonella,* yeasts, and molds. When epoxy-coated stainless steel strips identical to those used in juice tanks were inoculated with *Eurotium, Penicillium, Candida,* and *Saccharomyces cerevisiae* at populations >4 log CFU per

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area (2.5 by 7 cm) followed by treatment with ClO_2 gas, inoculum populations were reduced below the limit of detection *(20).*

The objective of the present study was to evaluate ClO_2 gas for its effectiveness in killing *Salmonella enterica, E. coli* O157:H7, and *L. monocytogenes* inoculated onto the surfaces of fresh-cut cabbage, carrot, and lettuce and its effectiveness in killing *Salmonella,* yeasts, and molds on the surfaces of fresh apples, tomatoes, onions, and peaches.

MATERIALS AND METHODS

Bacteria used and maintenance of cultures. Five-serotype or five-strain mixtures of each of three pathogens were prepared for inoculating produce. By using mixtures, the serotype or strain most resistant to ClO_2 was tested. This approach is preferable to using only one serotype or strain that may happen to be more sensitive to ClO_2. Isolates of *S. enterica* from alfalfa sprouts (serotype Agona), human feces of patients in two tomato-associated outbreaks (serotypes Baildon and Montevideo), orange juice (serotype Gaminara), and a cantaloupe-associated outbreak (serotype Michigan) were used. Five strains of *E. coli* O157:H7 isolated from a patient in a cider-associated outbreak (C7927), calf feces (E0018), and patients in outbreaks associated with alfalfa sprouts (F4546), lettuce (H1730), and unpasteurized apple cider (SEA 13B88) were used. Strains of *L. monocytogenes* were isolated from celery (serotype 4b, F8027), peach and plum (serotype 1/2b, F8255), corn (serotype 1/2a, F8369), a patient in a coleslaw-associated outbreak (serotype 4b, G1091), and potato (serotype 1/2a, H0222).

All serotypes of *Salmonella* and strains of *E. coli* O157:H7 were grown in tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks, Md.) supplemented with nalidixic acid (Sigma, St. Louis, Mo.) (TSBN) at a concentration of 50 μg/ml at 37°C for 24 h. *L. monocytogenes* strain was grown in brain heart infusion (BHI) broth (Difco, Becton Dickinson) supplemented with nalidixic acid (BHIN) at a concentration of 50 μg/ml at 37°C for 24 h. Cultures were combined with sterile glycerol (85:15, vol/vol, culture:glycerol) and stored as stock cultures at −20°C until used.

Preparation of inocula. Salmonellae and *E. coli* O157:H7 were grown on tryptic soy agar (pH 7.3) (TSA; BBL, Becton Dickinson) supplemented with nalidixic acid (50 μg/ml) and sodium pyruvate (100 μg/ml) (TSANP) at 37°C for 24 h. *L. monocytogenes* was grown on BHI agar (pH 7.4) (BHIA; Difco, Becton Dickinson) supplemented with nalidixic acid (50 μg/ml) and sodium pyruvate (100 μg/ml) (BHIANP) at 37°C for 48 h. Cells from single colonies of *Salmonella* and *E. coli* O157:H7 were inoculated into 10 ml of TSBN, and *L. monocytogenes* was inoculated into 10 ml of BHIN. A minimum of two consecutive 24-h transfers were made via loop inoculum (ca. 10 μl) into 10 ml of TSBN or BHIN. Cells were harvested by centrifugation at 2,000 × *g* for 15 min (Centra CL2 centrifuge, International Equipment Co., Needham Heights, Mass.) and resuspended in 5 ml of sterile 5% (vol/vol) horse serum (Sigma). Horse serum was used as a carrier for cells in an attempt to mimic the presence of organic material in inoculum that might contaminate the surface of produce. Suspensions of each serotype or strain of pathogen were combined to give 20 ml of a five-serotype mixture of *Salmonella,* 20 ml of a five-strain mixture of *E. coli* O157:H7, or 20 ml of a five-strain mixture of *L. monocytogenes,* each mixture containing approximately equal populations of each serotype or strain. Populations of *S. enterica* and *E. coli* O157:H7 in suspensions were determined by serially diluting suspensions in sterile 0.1% peptone and surface plating duplicate 0.1-ml samples on TSANP; the population of *L. monocytogenes* was determined by plating the diluted suspension on BHIANP. Plates were incubated at 37°C for 24 h (TSANP) or 48 h (BHIANP) before colonies were counted.

Fresh-cut vegetables tested. Cabbage (*Brassica oleracea* L. var. *capitata* L.), carrot (*Daucus carota* subsp. *sativus*), and iceberg lettuce (*Lactuca sativa* L.) were purchased at a produce market in Griffin, Ga., and stored at 4°C for a maximum of 2 days before being used in experiments. Prior to inoculation with *Salmonella, E. coli* O157:H7, or *L. monocytogenes,* two to four outer leaves of the heads of cabbage and lettuce were removed and discarded. The skin of carrots was removed with a peeler. Inner leaves of cabbage were cut into pieces (ca. 4 cm by 4 mm). Inner leaves of lettuce were cut into pieces (ca. 4 by 4 cm) similar in size to those commercially marketed as packaged salads. Carrots were cut with a knife into julienne style pieces (ca. 5 cm by 3 mm by 2 mm), also to resemble pieces used in packaged salad mixes. Twenty grams of cabbage, carrot, and lettuce was separately placed in single layers in plastic weigh trays (14 by 14 by 2.5 cm) in preparation for inoculation.

Fresh produce tested. Golden Delicious apples (*Malus domestica* Borkh.), tomatoes (*Lycopersicon esculentum* L.), Vidalia cv. onions (*Allium cepa* L.), and peaches (*Prunus persica*) were purchased at a produce market in Griffin, Ga., and stored at 22°C for a maximum of 1 day before being used in experiments. None of the produce used in the study was waxed or oiled. Samples consisting of one apple (ca. 150 ± 10 g) placed stem up, one tomato (215 ± 15 g) placed stem up, one onion (180 ± 25 g) placed root down, and one peach (103 ± 17 g) placed stem down were positioned in separate plastic weigh trays (14 by 14 by 2.5 cm) in preparation for inoculation.

Inoculation of produce. Fresh-cut vegetables at 22 ± 1°C in plastic weigh trays were spot inoculated with 100 μl of a five-serotype or five-strain mixture of *Salmonella, E. coli* O157:H7, or *L. monocytogenes* using a micropipette. Fresh produce tempered at 22 ± 1°C was used to simulate a temperature condition in field operations. Produce in plastic weigh trays was spot inoculated with 100 μl of a five-serotype mixture of *Salmonella.* For fresh-cut vegetables, inoculum was deposited on the cut tissue and intact tissue surface, and for whole produce, inoculum was deposited on the intact skin surface. To prevent inoculum from passing through the fresh-cut vegetables or running off the side of uncut produce and to facilitate drying, small approximately equal volumes of inoculum were applied at up to 10 locations on each sample. All produce was inoculated in a biosafety hood. The fresh-cut vegetables were dried for 30 min at 22 ± 2°C and then stored in plastic containers at 80% relative humidity for 22 h at 4°C before treatment with ClO_2 gas. Just prior to treatment, fresh-cut vegetables were placed in a biosafety hood for 30 min at 22 ± 1°C. After inoculation of whole produce, samples were stored in a biosafety hood for 20 to 22 h at 22 ± 1°C prior to treatment with ClO_2 gas.

Relative humidity. Three inoculated samples of either a fresh-cut vegetable or whole fruit or vegetable were placed in a transparent Plexiglas desiccator cabinet (45.7 by 30.5 by 30.5 cm; Fisher Scientific, Pittsburgh, Pa.). Samples were placed on the bottom three shelves of the four-shelf cabinet. High (62 to 98%) relative humidity was achieved by placing 20 ml of hot water (initially at 98 to 99°C) in a shallow plastic dish (8.6 by 8.6 by 2.2 cm) on the bottom shelf. A brushless 12VDC 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 thermohygro

recorder (model 11-661-13, Fisher) was used to monitor relative humidity and temperature inside the treatment cabinet.

ClO_2 gas treatment. Fresh-cut vegetables (one sample per shelf) inoculated with *Salmonella* at ca. 6.9 log CFU/g, *E. coli* O157:H7 at ca. 6.7 log CFU/g, or *L. monocytogenes* at ca. 6.7 log CFU/g and whole produce (one sample per shelf) inoculated with *Salmonella* at ca. 8.0 log CFU per piece were placed in the cabinet and treated with either air (control) or ClO_2 gas at 22 ± 1°C. Chemical sachets (ca. 9 by 18 cm; ICA TriNova, Inc., Marietta, Ga.) consisted of two compartments: one containing a granular porous solid impregnated with sodium chlorite and the other containing a granular porous solid impregnated with acid and an acid precursor (ferric chloride). Breakage of the septum between the two compartments followed by mixing of the chemicals initiated the production and release of ClO_2 gas into the treatment cabinet. The mixture of chemicals in three sachets was formulated to release ClO_2 gas into the cabinet (31.1 liters) at concentrations of 1.4, 2.7, and 4.1 mg/liter within 5.4 to 10.5, 10.4 to 20.0, and 20.5 to 30.8 min, respectively, at 23 ± 1°C. Because gas phase concentration of 1 mg/liter is equivalent to 362 ppmv, an alternative way to report the concentrations of ClO_2 gas released is 507, 977, and 1,484 ppmv within 5.4 to 10.5, 10.4 to 20.0, and 20.0 to 30.8 min, respectively.

The amounts of ClO_2 gas released into the treatment cabinet were quantified through a series of titrations of potassium iodide buffer. Procedures for analysis *(3)* were followed. A description of chemical reactions was provided by Aieta et al. *(2)*. Immediately following placement of the produce samples on the bottom three shelves, hot water (20 ml) was placed on the bottom shelf and three sachets containing the reactant chemicals were simultaneously placed on an elevated mesh platform on the top shelf to deliver elevated levels of relative humidity and desired ClO_2 gas concentrations. The control samples were handled in an identical manner, except ClO_2 sachets were not placed in the cabinet. The cabinet was sealed by closing and securing the door to which a rubber gasket was affixed.

Microbiological analyses. Uninoculated and inoculated samples of fresh-cut vegetables and whole fruits and vegetables not exposed to air (control) or ClO_2 gas treatment in the cabinet and samples held for up to 30.8 min in air or ClO_2 gas in the cabinet were analyzed for populations of *Salmonella*, *E. coli* O157:H7, *L. monocytogenes*, yeasts, and molds. Untreated and treated fresh-cut vegetables were placed in separate Stomacher 400 bags (Seward Medical Ltd., London, UK), and 100 ml of Dey-Engley (DE) broth (pH 7.6) (Difco, Becton Dickinson) was immediately added to each bag. Samples were pummeled at normal speed for 1 min in a Stomacher 400 blender. Untreated and treated apples, tomatoes, onions, and peaches were individually placed in quart-size Ziploc bags, and 20 ml of DE broth was added. Samples were gently hand rubbed in DE broth for 1 min.

Undiluted DE broth from homogenates of fresh-cut vegetables or DE broth used to wash uncut produce (0.25 ml in quadruplicate and 0.1 ml in duplicate) and samples (0.1 ml in duplicate) serially diluted in sterile 0.1% peptone were surface plated on TSANP to enumerate *Salmonella* or *E. coli* O157:H7 and on BHIANP to enumerate *L. monocytogenes*. Samples from whole produce were surface plated on dichloran rose bengal chloramphenicol (DRBC) agar (pH 5.6) (Difco, Becton Dickinson) to enumerate yeasts and molds. The TSANP plates were incubated at 37°C for 24 h before presumptive positive *Salmonella* or *E. coli* O157:H7 colonies were counted. Five to 10 presumptive positive *Salmonella* colonies from each sample were randomly selected for confirmation using a *Salmonella* latex test (Oxoid, Basingstoke, UK), lysine iron agar (pH 6.7) (Difco, Becton Dickinson), and triple sugar iron agar (Difco, Becton Dickinson). Five to 10 presumptive positive *E. coli* O157:H7 colonies were randomly selected for confirmation using an *E. coli* O157 latex test (Oxoid). The BHIANP plates were incubated at 37°C for 48 h before presumptive positive *L. monocytogenes* colonies were counted. Five to 10 presumptive positive *L. monocytogenes* colonies from each sample were randomly selected for confirmation using API *Listeria* diagnostic kit (bioMérieux Vitek Inc., Hazelwood, Mo.). The DRBC agar plates were incubated at 25°C for 5 days before yeast and mold colonies were counted.

Following removal of homogenates from bags containing fresh-cut vegetables and DE wash broth from bags containing whole produce for direct plating to enumerate pathogens and yeasts and molds, 100 ml of 2× lactose broth supplemented with nalidixic acid (100 μg/ml) and sodium pyruvate (200 μg/ml) (LBNP) was added to each bag containing DE wash broth and fresh-cut vegetables inoculated with *Salmonella*, 100 ml of 2× modified tryptic soy broth (mTSB) *(34)* (pH 7.0) supplemented with nalidixic acid (100 μg/ml) and sodium pyruvate (200 μg/ml) (mTSBNP) was added to each bag containing DE wash broth and fresh-cut vegetables inoculated with *E. coli* O157:H7, and 100 ml of 2× *Listeria* enrichment broth (LEB) supplemented with nalidixic acid (20 μg/ml) and sodium pyruvate (200 μg/ml) (LEBNP) (pH 7.3) (Oxoid) was added to each bag containing DE wash broth and fresh-cut vegetables inoculated with *L. monocytogenes*. To each bag containing DE wash broth and whole produce, 200 ml of 1× LBNP was added. Mixtures of either fresh-cut vegetables or uncut produce with DE broth and LBNP, mTSBNP, or LEBNP were incubated, respectively, at 37°C for 24 h to preenrich for *Salmonella* and enrich for *E. coli* O157:H7 or at 37°C for 48 h to enrich for *L. monocytogenes*. When samples did not yield one or more colonies of *Salmonella* or *E. coli* O157:H7 on TSANP or one or more colonies of *L. monocytogenes* on BHIANP, preenriched or enriched mixtures were examined for the presence of pathogens. A loop (ca. 10 μl) of each DE broth–produce–LBNP preenrichment mixture was streaked onto xylose lysine desoxycholate (XLD; Difco, Becton Dickinson) agar supplemented with nalidixic acid (50 μg/ml) and sodium pyruvate (100 μg/ml) (XLDNP, pH 7.4) for isolation of *Salmonella*, and the DE broth–produce–mTSBNP mixture was streaked on sorbitol MacConkey (SMAC) agar (Difco, Becton Dickinson) supplemented with nalidixic acid (50 μg/ml) and sodium pyruvate (100 μg/ml) (SMACNP, pH 7.1) for isolation of *E. coli* O157:H7. The DE broth–produce–LEBNP mixture was streaked onto modified Oxford (MOX; Oxoid) agar supplemented with nalidixic acid (50 μg /ml) and sodium pyruvate (100 μg/ml) (MOXNP, pH 7.0) for isolation of *L. monocytogenes*. Plates were incubated at 37°C for 24 h before being examined for the presence of presumptive *Salmonella* or *E. coli* O157:H7 colonies or at 37°C for 48 h before being examined for presumptive *L. monocytogenes* colonies. Colonies were subjected to confirmation tests as described above. In addition, 0.1-ml samples of the prechenriched mixture in LBNP were inoculated into 10 ml of Rappaport-Vassiliadas enrichment broth (pH 5.1; Difco, Becton Dickinson), which was incubated at 42°C for 24 h and streaked on XLDNP agar if samples of preenriched mixtures did not yield *Salmonella* colonies on the XLDNP agar plates. XLDNP agar on which enriched samples were streaked was incubated at 37°C for 24 h before being examined for presumptive *Salmonella* colonies, followed by confirmation.

Sensory evaluation. An untrained panel of 18 to 21 technicians and graduate students in the Center for Food Safety and Department of Food Science and Technology of the University of Georgia evaluated apples and fresh-cut vegetables treated with 1.4

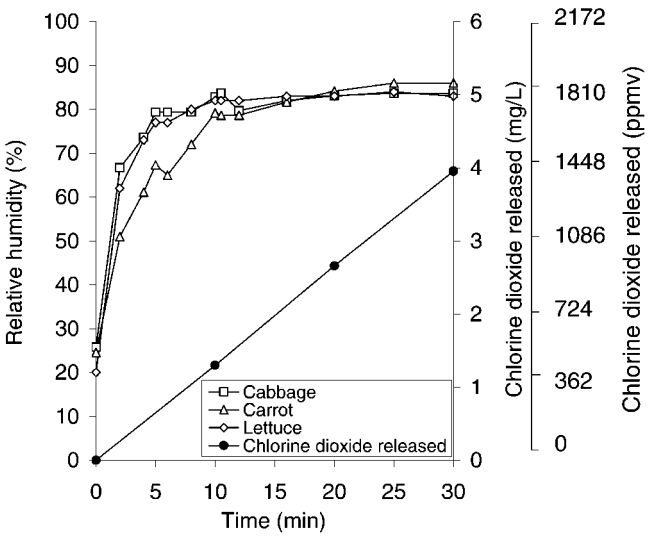

FIGURE 1. *Atmospheric relative humidity (□, △, ◇) in the cabinet housing fresh-cut cabbage, carrot, and lettuce, respectively, that had been inoculated with* Salmonella *and treated with ClO_2 gas (●) released into the cabinet.*

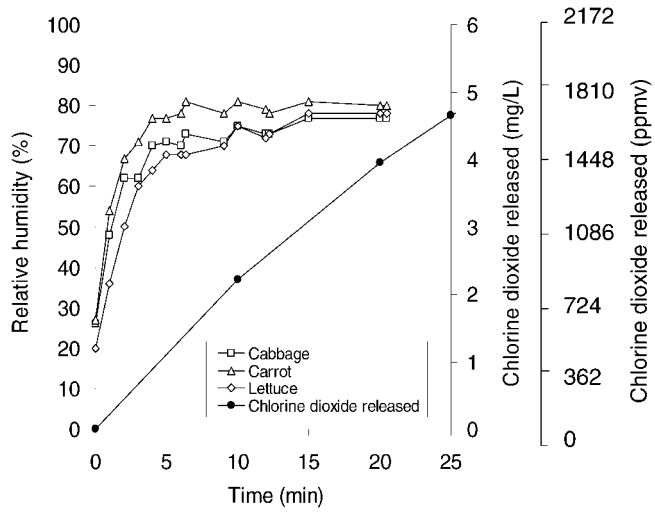

FIGURE 2. *Atmospheric relative humidity (□, △, ◇) in the cabinet housing fresh-cut cabbage, carrot, and lettuce, respectively, that had been inoculated with* E. coli *O157:H7 and treated with ClO_2 gas (●) released into the cabinet.*

mg/liter ClO_2, tomatoes treated with 2.7 mg/liter ClO_2, and onions and peaches treated with 4.1 mg/liter ClO_2. These treatment concentrations were selected for sensory evaluation based on observations from microbiological studies. Treatment concentrations reduced populations of pathogens without causing immediate substantive changes in sensory quality, as assessed by subjective evaluation. Produce used for these studies was not inoculated with pathogens.

Treated and untreated (control) fresh-cut cabbage, carrots, and lettuce (20-g samples) were placed in bags measuring 16 by 16 cm (CP930 film, 1.75 mil, OTR 300 $cm^3/100\ in^2/24$ h; Cryovac, Inc., Duncan, S.C.), heat sealed, and stored at 10°C for up to 10 days before being evaluated for sensory quality. Treated and untreated apples, tomatoes, onions, and peaches were held at 21°C for up to 41, 10, 31, and 10 days, respectively, before sensory quality was evaluated. For fresh-cut cabbage, carrot, and lettuce in each of three replicate experiments, one treated sample and one untreated sample were presented to panelists in weigh boats marked with random three-digit numbers. For apples, tomatoes, onions, and peaches, each of three replicate experiments consisted of presenting three treated and three untreated samples to panelists. Panelists were asked to carefully examine the produce for appearance, color, aroma, and overall quality. Sensory attributes were rated by assigning scores of 1 through 9 on a 9-point hedonic scale, with 1 = dislike extremely, 5 = neither like nor dislike, and 9 = like extremely. All evaluations were conducted within 1 h after treating the samples with ClO_2 gas (day 0) or within 1 h of removing the samples from storage at 10 or 21°C for up to 41 days.

Statistical analyses. All experiments were replicated three times. Each replicate experiment involving inoculated produce consisted of three samples exposed to the same treatment conditions. Mean values were analyzed to determine significant differences ($\alpha = 0.05$) in populations of *Salmonella, E. coli* O157:H7, *L. monocytogenes,* and yeasts and molds recovered from produce subjected to treatment with air (control) or various concentrations of ClO_2. Each replicate experiment involving evaluation of sensory qualities of produce consisted of two or three samples exposed to the same treatment. Mean values were analyzed to determine significant differences ($\alpha = 0.05$) in sensory ratings as affected by treatment and storage time. Data were subjected to analysis of variance and Duncan multiple range tests (SAS Institute, Cary, N.C.).

RESULTS AND DISCUSSION

Treatment of fresh-cut cabbage. Fresh-cut cabbage inoculated with *Salmonella, E. coli* O157:H7, or *L. monocytogenes* was treated with ClO_2 gas at release concentrations of 1.4, 2.7, and 4.1 mg/liter within 6.4 to 10.5, 12.3 to 20.0 and 20.5 to 30.8 min, respectively, at 48 to 85% relative humidity and 22 ± 1°C (Figs. 1, 2, and 3, respectively). Log reductions of pathogens resulting from these treatments are shown in Table 1. Compared with cabbage treated with air (control), treatments with all test concentrations of ClO_2 caused significant reductions ($\alpha = 0.05$)

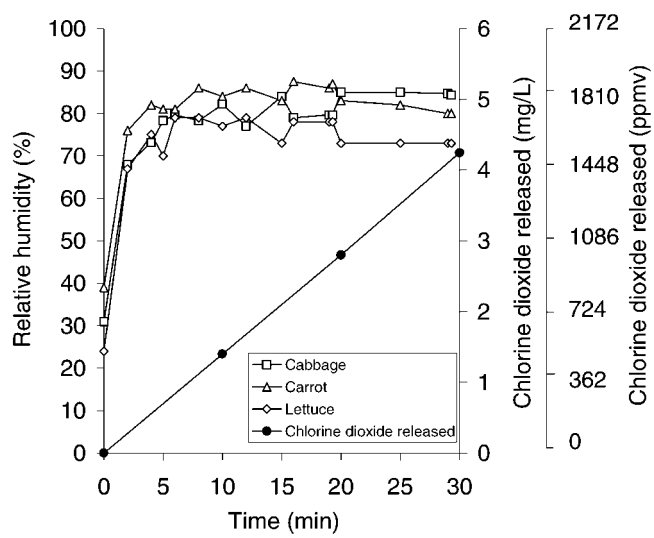

FIGURE 3. *Atmospheric relative humidity (□, △, ◇) in the cabinet housing fresh-cut cabbage, carrot, and lettuce, respectively, that had been inoculated with* L. monocytogenes *and treated with ClO_2 gas (●) released into the cabinet.*

TABLE 1. *Recovery of pathogens from fresh-cut cabbage, carrot, and lettuce treated with ClO_2 gas*

Pathogen	Vegetable	Treatment time (min)	Amount of ClO_2 released (mg/liter)	Population (log CFU/g)[a]		
				Recovered[b]	Reduction[c]	Enrichment[d]
Salmonella	Cabbage	0	0	6.85 A		
		10.5	1.4	5.61 B	1.24	
		20.0	2.7	4.96 B	1.89	5/5
		30.8	4.1	2.43 B	4.42	
	Carrot	0	0	7.00 A		
		10.5	1.4	4.95 B	2.15	
		20.0	2.7	3.99 B	3.11	1/4
		30.8	4.1	1.95 B	5.15	
	Lettuce	0	0	6.81 A		
		10.5	1.4	5.68 B	1.14	
		20.0	2.7	5.58 B	1.21	2/3
		30.8	4.1	5.26 B	1.58	
E. coli O157:H7	Cabbage	0	0	6.78 A		
		6.4	1.4	5.25 B	1.53	
		12.3	2.7	4.10 B	2.68	
		20.5	4.1	3.65 B	3.13	1/1
	Carrot	0	0	6.81 A		
		6.4	1.4	4.78 B	2.03	
		12.3	2.7	3.63 B	3.18	2/2
		20.5	4.1	1.19 B	5.62	8/8
	Lettuce	0	0	6.73 A		
		6.4	1.4	6.09 B	0.64	
		12.3	2.7	6.01 B	0.72	
		20.5	4.1	5.16 C	1.57	4/4
L. monocytogenes	Cabbage	0	0	6.71 A		
		10.0	1.4	4.95 B	1.76	
		19.3	2.7	3.40 B	3.31	1/1
		29.3	4.1	3.11 B	3.60	3/3
	Carrot	0	0	6.48 A		
		10.0	1.4	3.20 B	3.28	1/1
		19.3	2.7	1.13 B	5.35	4/8
		29.3	4.1	0.60 B	5.88	1/9
	Lettuce	0	0	6.41 A		
		10.0	1.4	5.60 B	0.81	
		19.3	2.7	5.18 B	1.23	1/1
		29.3	4.1	4.88 B	1.53	0/2

[a] Populations of pathogens recovered in TSANP after treatment of cabbage, carrot, and lettuce with 0, 1.4, 2.7, and 4.1 mg/liter ClO_2. Initial populations inoculated on cabbage, carrot, and lettuce were 6.96, 7.00, and 6.86 log CFU/g, respectively, for *Salmonella*, 6.72, 6.72, and 6.64 log CFU/g, respectively, for *E. coli* O157:H7, and 6.75, 6.80, and 6.69 log CFU/g, respectively, for *L. monocytogenes*. The detection limit was 1 CFU/ml of DE wash (5 CFU/g of cabbage, carrot, or lettuce).

[b] Within each pathogen and vegetable, mean values with different letters are significantly different ($\alpha = 0.05$).

[c] Reduction (log CFU/g) compared with the number recovered from cabbage, carrot, or lettuce receiving no ClO_2 treatment (0 mg/liter).

[d] Number of samples positive for test pathogen/number of samples analyzed of treated, homogenized fresh-cut vegetables as detected by enrichment. Samples on which pathogens were recovered by direct plating were not analyzed by enrichment.

in the number of viable cells of test pathogens. Highest log reductions for *Salmonella* (4.42 log CFU/g), *E. coli* O157:H7 (3.13 log CFU/g), and *L. monocytogenes* (3.60 log CFU/g) resulted from treatment with 4.1 mg/liter ClO_2. With the exception of *E. coli* O157:H7 on lettuce treated with 4.1 mg/liter ClO_2, reductions achieved using 1.4, 2.7, or 4.1 mg/liter ClO_2 were not significantly different ($\alpha = 0.05$) within each pathogen and vegetable. Large standard deviations in the data resulted in a lack of significant differences in the numbers of each pathogen killed by treatment with 1.4 to 4.1 mg/liter ClO_2, although trends toward significantly higher reductions are evident with increased treatment concentrations. Differences in the number of pathogens cells that survived treatment with various concentrations of ClO_2 also are evident in enrichment data. The percentage of positive samples after enrichment was not necessarily correlated with counts obtained by direct plating of companion samples receiving the same treatment. The same trends were found for other fresh-cut and whole produce. Compared with log reductions achieved by treatment of fresh-cut carrots, reductions in fresh-cut cabbage were 1 to 2 log CFU/g lower. This difference may be due in part to the generally lower relative humidity in the chamber during cabbage treatment compared with that during carrot

TABLE 2. *Mean hedonic ratings for sensory attributes of fresh-cut vegetables treated with 1.4 mg/liter ClO_2*

Vegetable	Storage time at 10°C (days)	Amount of ClO_2 released (mg/liter)	Ratings for sensory attributes[a]: Appearance	Color	Aroma	Overall quality
Cabbage	0	0	A 7.3 X	A 7.3 X	A 6.5 X	A 7.2 X
		1.4	A 5.2 Y	A 5.0 Y	A 4.7 Y	A 4.9 Y
	3	0	B 6.0 X	B 6.0 X	B 5.3 X	B 6.0 X
		1.4	B 4.2 Y	B 4.2 Y	AB 4.8 Y	B 4.2 Y
	7	0	D 4.6 X	D 4.4 X	D 4.7 X	D 4.4 X
		1.4	C 2.7 Y	C 2.7 Y	B 4.4 Y	C 2.8 Y
	10	0	C 5.1 X	C 5.0 X	C 5.1 X	C 5.0 X
		1.4	D 2.2 Y	D 2.1 Y	C 3.6 Y	D 2.2 Y
Carrot	0	0	A 8.0 X	A 7.9 X	A 6.6 X	A 7.5 X
		1.4	A 6.8 Y	A 6.8 Y	A 5.8 Y	A 6.2 Y
	3	0	B 7.3 X	B 7.5 X	B 6.3 X	A 7.4 X
		1.4	B 5.3 Y	B 5.1 Y	A 5.7 Y	B 5.2 Y
	7	0	C 6.9 X	C 7.0 X	C 5.9 X	B 6.8 X
		1.4	B 5.2 Y	B 5.1 Y	A 5.4 X	B 5.0 Y
	10	0	C 6.6 X	D 6.7 X	D 5.3 X	C 6.0 X
		1.4	B 5.0 Y	B 5.0 Y	A 5.4 Y	B 4.8 Y
Lettuce	0	0	A 7.8 X	A 7.8 X	A 6.8 X	A 7.6 X
		1.4	A 6.0 Y	A 5.9 Y	A 5.7 Y	A 5.9 Y
	3	0	B 6.0 X	B 6.1 X	B 5.7 X	B 6.1 X
		1.4	B 2.9 Y	B 2.9 Y	B 4.3 Y	B 3.0 Y
	7	0	B 5.6 X	B 5.6 X	C 5.1 X	C 5.4 X
		1.4	C 2.4 Y	B 2.3 Y	C 3.8 Y	C 2.4 Y
	10	0	C 2.7 X	C 2.7 X	D 3.8 X	D 2.8 X
		1.4	C 2.2 Y	C 2.1 Y	D 3.1 Y	C 2.2 Y

[a] Ratings were assigned by panelists using a 9-point hedonic scale: 1 = dislike extremely, 5 = neither like nor dislike, and 9 = like extremely. Within the same vegetable, treatment, and attribute, mean values not preceded by the same letter are significantly different ($\alpha = 0.05$) with respect to storage time and mean values not followed by the same letter are significantly different ($\alpha = 0.05$) with respect to ClO_2 concentration.

treatment, which would retard the lethality of ClO_2, and in part to differences in surface tissues of the two vegetables.

Subjective examination of fresh-cut cabbage revealed very slight browning after treatment with 2.7 mg/liter ClO_2 for 12.3 to 20.0 min at 73 to 83% relative humidity. Cabbage treated with 4.1 mg/liter ClO_2 at 77 to 84% relative humidity had more brown discoloration, a condition resulting from biochemical reactions of ClO_2 with tissue components. Mean ratings for sensory attributes of fresh-cut cabbage treated with 1.4 mg/liter ClO_2, which resulted in only 1.24 to 1.76 log CFU/g reductions in pathogens, are shown in Table 2. Treatment caused immediate significant ($\alpha = 0.05$) decreases in appearance, color, aroma, and overall sensory quality. The overall quality of treated cabbage was rated 2.8 on day 7, which falls in the ''dislike very much'' to ''dislike moderately'' range on the 9-point hedonic scale. Brown discoloration was a major contributor to the deterioration of sensory qualities.

Treatment of fresh-cut carrots. Julienne-style cut carrots inoculated with *Salmonella*, *E. coli* O157:H7, or *L. monocytogenes* were treated with ClO_2 at concentrations of 1.4, 2.7, and 4.1 mg/liter within 6.4 to 10.5, 12.3 to 20, and 20.5 to 30.8 min, respectively, at 51 to 88% relative humidity and 22 ± 1°C (Figs. 1 through 3). Populations of the three pathogens recovered from untreated and treated carrots are given in Table 1. Inoculated fresh-cut carrots treated with 1.4 mg/liter ClO_2 had significant reductions ($\alpha = 0.05$) in populations of *Salmonella*, *E. coli* O157:H7, and *L. monocytogenes* of 2.15, 2.03, and 3.28 log CFU/g, respectively. Larger but not significantly different reductions were caused by treatment with 2.7 and 4.1 mg/liter ClO_2 compared with treatment with 1.4 mg/liter. The highest reductions in population (5.15 log CFU/g for *Salmonella*, 5.62 log CFU/g for *E. coli* O157:H7, and 5.88 log CFU/g for *L. monocytogenes*) resulted from treatment of carrots with 4.1 mg/liter ClO_2.

The effectiveness of ClO_2 gas for killing all three pathogens on fresh-cut carrots may in part reflect an additive or synergistic effect caused by natural antimicrobials present in carrot juice. Studies have revealed a lack of growth and even a reduction in populations of pathogens inoculated into carrot juice and onto fresh-cut carrots. Beuchat and Brackett *(6)* reported that raw carrot juice is lethal to *L. monocytogenes.* Abdul-Raouf et al. *(1)* observed that the number of *E. coli* O157:H7 cells on inoculated shredded carrots stored at 5°C for 14 days decreased by 1 log CFU/g within 3 days and by 5 log CFU/g within 7 days of storage. In this same study, *E. coli* O157:H7 inoculated on sliced cucumber followed by storage at 5°C was reduced by only 1.67 log CFU/g after 14 days of storage, suggesting that components unique to carrots may be responsible for

pathogen death. According to Abdul-Raouf et al. *(1)* and Kurosaki and Nishi *(28)*, 6-methyoxymellein, a phytoalexin in carrot, inhibits the growth of several fungi and bacteria. This and perhaps other phytoalexins may also have been inhibitory or toxic to *Salmonella, E. coli* O157:H7, and *L. monocytogenes* inoculated onto fresh-cut carrots in our study.

Subjective evaluation of carrots after treatment with 1.4 mg/liter ClO_2 for 6.4 to 10.5 min at 79 to 84% relative humidity revealed a slight whitening in color (Table 2). Higher concentrations of ClO_2 (2.7 and 4.1 mg/liter) applied at 12.3 to 20.0 and 20.5 to 30.8 min at 78 to 87% and 80 to 85% relative humidity, respectively, depending on the test pathogen, caused more whitening. Treatment of fresh-cut carrots with 1.4 mg/liter ClO_2 caused a significant decrease ($\alpha = 0.05$) in ratings assigned by the sensory panel. Ratings for appearance, color, aroma, and overall quality were in the 5 to 6 (''neither like nor dislike'' to ''like slightly'') range on the 9-point hedonic scale within 3 days. Although reductions in pathogen populations of 2.03 to 3.28 log CFU/g (Table 1) resulted from treatment with 1.4 mg/liter ClO_2, adverse effects on sensory quality render its application to carrots on a commercial scale questionable.

Treatment of fresh-cut lettuce. Fresh-cut lettuce inoculated with *Salmonella, E. coli* O157:H7, or *L. monocytogenes* was treated with ClO_2 at concentrations of 1.4, 2.7, and 4.1 mg/liter within 6.4 to 10.5, 12.3 to 20, and 20.5 to 30.8 min, respectively, at 36 to 84% relative humidity and 22 ± 1°C (Figs. 1 through 3). Reductions in populations of pathogens are given in Table 1. Treatment with ClO_2 at 1.4 mg/liter significantly reduced ($\alpha = 0.05$) the number of *Salmonella, E. coli* O157:H7, and *L. monocytogenes* by 1.14, 0.64, and 0.81 log CFU/g, respectively, compared with populations on untreated fresh-cut lettuce. There were no significant differences in the log reductions in *Salmonella* and *L. monocytogenes* on lettuce treated with the three concentrations of ClO_2. However, for lettuce inoculated with *E. coli* O157:H7, the highest concentration (4.1 mg/liter ClO_2) resulted in the highest reduction (1.57 log CFU/g), which was significantly different than that for treatments with 1.4 and 2.7 mg/liter ClO_2 or for untreated control.

Reductions in populations of the three pathogens on fresh-cut lettuce were substantially lower than those reported on lettuce leaves by Lee et al. *(29)*, who achieved reductions of 4.3, 3.4, and 5.0 log CFU/g for *Salmonella* Typhimurium, *E. coli* O157:H7, and *L. monocytogenes*, respectively, by treating lettuce leaves with ClO_2 at 4.3 mg/liter. The presence of test pathogens in juice released by the cut lettuce in our experiment may have provided protection against contact with ClO_2 gas, thereby resulting in higher numbers of cells surviving treatments. The lower reductions in populations of pathogens on fresh-cut lettuce compared with reductions on fresh-cut cabbage or fresh-cut carrots treated with the same concentrations of ClO_2 in our study also may be due in part to protection of bacteria afforded by their location in inaccessible sites in the cut tissue and in stomata on the uncut surface, a phenomenon also noted by Garg et al. *(18)*. Cut tissue facilitates microbial infiltration and exposes juices that would have a neutralizing effect on ClO_2 activity and provide an environment for growth of microorganisms *(8, 9)*.

Rodgers et al. *(36)* inoculated whole and sliced apples, uncut and shredded lettuce, strawberries, and cantaloupe with *L. monocytogenes* and *E. coli* O157:H7 and then treated these vegetables with a ClO_2 solution (3 and 5 μg/ml) for 5 min. Pathogens were recovered from shredded lettuce and sliced apples, supporting the observation that increased exposure of ClO_2 to organic materials resulting from breakage of cellular structures caused by cutting, slicing, or shredding offers protection against inactivation. Abdul-Raouf et al. *(1)* reported that *E. coli* O157:H7 survived in higher numbers on shredded lettuce than on sliced cucumbers stored at 5°C. Retention of higher numbers of *Salmonella, E. coli* O157:H7, and *L. monocytogenes* on treated fresh-cut lettuce than on treated fresh-cut cabbage in our study may indicate that differences in surface structure of the two vegetables and factors associated with tissue juice components influence the susceptibility of pathogens to potentially lethal effects of ClO_2. Other researchers have found a preference of microorganisms to initially attach to and enter stomata, broken trichomes, cracks in the cuticle, and the cut edges of lettuce *(38)* and other produce *(9)*, thereby resulting in protection against sanitizers. Visualization of infiltration has been achieved using confocal scanning laser microscopy. Seo and Frank *(38)* inoculated lettuce with *E. coli* O157:H7 and then treated the lettuce with 20 mg/liter chlorine for 5 min. Viable cells of the pathogen were observed entrapped 20 to 100 μm below the leaf surface in stomata and on cut edges, but cells on the surface of the leaves were dead. Harborage of *E. coli* O157:H7 and *Salmonella* in subsurface tissues of apple has also been described *(26, 27)*.

Immediately following treatment with 1.4 mg/liter ClO_2 for 6.4 to 10.5 min at 68 to 82% relative humidity, subjective evaluation revealed that fresh-cut lettuce underwent slight browning. Treatment with higher concentrations (2.7 and 4.1 mg/liter ClO_2 within 12.3 to 20.0 and 20.5 to 30.8 min, respectively) at 73 to 83% relative humidity resulted in more browning discoloration, although relative to fresh-cut carrots that received the same ClO_2 treatment, the degree of discoloration was lower. Results from the sensory panel evaluation of fresh-cut lettuce treated with 1.4 mg/liter ClO_2 and stored at 10°C for up to 10 days are shown in Table 2. As with cabbage and carrot, treatment had an immediate and significant adverse affect ($\alpha = 0.05$) on sensory quality. Ratings for appearance, color, and overall quality decreased to the ''dislike very much'' to ''dislike moderately'' range within 3 days. Overall, compared with observations on fresh-cut cabbage and carrot, visual sensory quality and viability of pathogens on fresh-cut lettuce were less affected by treatment with ClO_2 gas. Only modest reductions in pathogen populations (0.64 to 1.14 log CFU/g) were achieved by treating lettuce with 1.4 mg/liter ClO_2 (Table 1), making it less effective than several aqueous sanitizers known to kill higher numbers of pathogens on lettuce without compromising sensory quality.

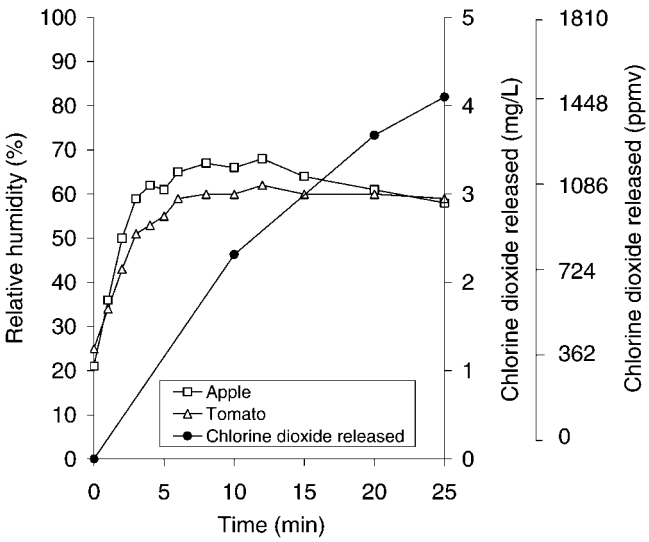

FIGURE 4. *Atmospheric relative humidity (□, △) in the cabinet housing whole apples and tomatoes, respectively, that were treated with ClO_2 gas (●) released into the cabinet.*

Treatment of apples. Fresh apples inoculated with *Salmonella* were treated with ClO_2 gas at concentrations of 1.4, 2.7, and 4.1 mg/liter within 6, 12, and 25 min, respectively, at 22 ± 1°C and 35 to 68% relative humidity (Fig. 4). Reductions in *Salmonella* populations are given in Table 3. Compared with untreated apples, populations of *Salmonella* recovered from apples treated with all test concentrations of ClO_2 were significantly lower ($\alpha = 0.05$), although they were not significantly different from each other. The same was true for yeasts and molds. The highest log reduction (4.21 log CFU per apple) in number of *Salmonella* cells was achieved by treatment with 2.7 and 4.1 mg/liter ClO_2, whereas treatment with 1.4 mg/liter ClO_2 caused a reduction of 3.21 log CFU per apple. These results are similar to those of Du et al. *(14)*, who found that apples inoculated with *E. coli* O157:H7 and treated with 1.1, 3.3, and 4.8 mg/liter ClO_2 gas for 10 min resulted in reductions of 2.8, 3.9, and 4.8 log CFU per spotted site, respectively. Treatment of apples with the same concentrations of ClO_2 for 20 min resulted in reductions of 4.7, 5.9, and ≥6.3 log CFU per spotted site, respectively. In another study on inactivation of *L. monocytogenes* on apples, treatment with 1.0, 3.0, and 4.0 mg/liter ClO_2 gas for 10 min caused reductions of 3.2, 3.3, and 5.5 log CFU per spotted site, respectively *(14)*. In our study, reductions in *Salmonella* populations as a result of treating apples with 1.4 mg/liter ClO_2 for 6 min or with 2.7 mg/liter ClO_2 for 12 min were slightly higher than reductions observed by Du et al. *(14, 15)* for *E. coli* O157:H7 and *L. monocytogenes* on apples treated with 1.1 to 3.3 mg/liter and 1.0 to 3.0 mg/liter ClO_2, respectively, for 10 min. Differences in these results can be attributed in part to differences in the resistance of test pathogens to ClO_2 and to differences in systems used to generate ClO_2 in different laboratories.

Yeast and mold populations detected on apples treated with 1.4, 2.7, and 4.1 mg/liter ClO_2 were significantly lower ($\alpha = 0.05$) than those on untreated apples. The highest log reduction (1.68 log CFU per apple) resulted from treatment with 4.1 mg/liter ClO_2. Compared with *Salmonella*, yeasts and molds naturally present on the skin of apples appear to be less susceptible to ClO_2 gas. Rodgers et al. *(36)* reported that yeast populations on apples treated with aqueous ClO_2 (3 and 5 μg/ml) were reduced by 1.3 to 1.5 log CFU/g, mold populations were reduced by 1.4 to 1.5 log CFU/g, and *L. monocytogenes* or *E. coli* O157:H7 populations were reduced by ca. 5 log CFU/g, indicating that ClO_2 gas is much more lethal to bacteria than to yeasts and molds. This finding is in agreement with the observation that mold propagules on apples tend to be more resistant than bacteria to sanitizers used in the apple industry *(7)*. Differences in sensitivity of microbial cells may also be influenced by their hydrophobicity. Waxes in cutin on the surface of apples contain alcohols, morpholine, and surfactants that may enhance dispersion of spores, thereby resulting in an apparent increased number of CFUs dispersed in wash solutions *(26)*.

Subjective evaluation revealed that treatment of apples with 4.1 mg/liter ClO_2 gas for 25 min at 58% relative humidity caused the formation of small brown spots on the skin. The appearance of apples treated with 1.4 and 2.7 mg/liter ClO_2 at 65 to 68% relative humidity was unaffected. Because treatment of apples with 1.4 mg/liter ClO_2 caused a 3.21 log CFU per apple reduction in *Salmonella* (Table 3), this treatment was selected to determine its effects on sensory qualities. Ratings assigned to apples stored at 21°C for up to 41 days are shown in Table 4. Although treated apples were consistently judged slightly but significantly ($\alpha = 0.05$) poorer in appearance, color, and overall quality, ratings did not fall below "neither like nor dislike" throughout the 41-day storage period. Treatment of apples with ClO_2 gas caused a substantial reduction in the population of *Salmonella* without markedly compromising sensory quality and thus deserves further evaluation on a commercial scale.

Treatment of tomatoes. Fresh tomatoes inoculated with *Salmonella* were treated with ClO_2 gas at concentrations of 1.4, 2.7, and 4.1 mg/liter within 6, 12, and 25 min, respectively, at 22 ± 1°C and 34 to 62% relative humidity (Fig. 4). Significant reductions ($\alpha = 0.05$) in populations of *Salmonella* resulted from treatment with all concentrations of ClO_2 (Table 3). The highest concentration, 4.1 mg/liter ClO_2, caused the highest log reduction (4.33 log CFU per tomato), although this reduction was not significantly different than those resulting from other ClO_2 treatments. Log reductions on tomatoes were lower than those on apples treated with 1.4 and 2.7 mg/liter ClO_2; however, treatment of apples and tomatoes with 4.1 mg/liter ClO_2 resulted in similar reductions. On tomatoes, the reduction of *Salmonella* (4.33 log CFU per tomato) as a result of treatment with 4.1 mg/liter ClO_2 gas for 25 min is similar to the reduction of *Salmonella* (3.67 log CFU/cm^2) after treatment for 30 min with neutral electrolyzed water (NEW) solution containing 89 mg/liter active chlorine *(11)*; however, ClO_2 gas has a greater kill capacity than NEW, requiring a lower

TABLE 3. *Recovery of* Salmonella *and yeasts and molds from produce treated with ClO_2 gas*

Produce	Microorganism	Treatment time (min)	Amount of ClO_2 released (mg/liter)	Population (log CFU/piece)[a]		
				Recovered[b]	Reduction[c]	Enrichment[d]
Apple	*Salmonella*	0	0	5.49 A		
		6	1.4	2.28 B	3.21	1/7
		12	2.7	1.28 B	4.21	1/9
		25	4.1	1.28 B	4.21	0/9
	Yeasts and molds	0	0	5.92 A		
		6	1.4	4.83 B	1.09	
		12	2.7	4.44 B	1.48	
		25	4.1	4.24 B	1.68	
Tomato	*Salmonella*	0	0	5.90 A		
		6	1.4	4.79 B	1.11	2/3
		12	2.7	3.86 B	2.04	0/5
		25	4.1	1.57 B	4.33	1/6
	Yeasts and molds	0	0	6.17 A		
		6	1.4	5.30 A	0.87	
		12	2.7	5.35 A	0.82	
		25	4.1	5.01 A	1.16	
Onion	*Salmonella*	0	0	6.95 A		
		5.4	1.4	6.12 B	0.83	
		10.4	2.7	5.06 B	1.89	
		20	4.1	5.01 B	1.94	
	Yeasts and molds	0	0	6.24 AB		
		5.4	1.4	5.88 B	0.36	
		10.4	2.7	6.02 B	0.22	
		20	4.1	6.46 A	+0.22	
Peach	*Salmonella*	0	0	7.19 A		
		5.4	1.4	6.19 B	1.00	
		10.4	2.7	5.67 B	1.52	2/2
		20	4.1	3.96 B	3.23	5/5
	Yeasts and molds	0	0	4.84 A		
		5.4	1.4	4.44 AB	0.41	
		10.4	2.7	2.43 B	2.41	
		20	4.1	2.60 B	2.65	

[a] Populations of *Salmonella* recovered on TSANP and yeasts and molds recovered on DRBC agar after treatment of produce at 22 ± 1°C. Populations of *Salmonella* inoculated on produce were 8.00 log CFU per apple, 8.00 log CFU per tomato, 7.96 log CFU per onion, and 8.03 log CFU per peach. The detection limit was 1 CFU/ml of DE wash (20 CFU per piece of produce).

[b] Within each type of produce and microorganism, mean values not followed by the same letter are significantly different ($\alpha = 0.05$).

[c] Reduction (log CFU per piece of produce) compared with the number recovered from produce receiving no ClO_2 treatment (0 mg/liter).

[d] Number of pieces positive for *Salmonella*/number of pieces analyzed of treated, washed produce as detected by enrichment. Produce on which *Salmonella* was recovered by direct plating was not analyzed by enrichment.

concentration to achieve approximately the same log reductions as treatment with NEW.

Yeast and mold populations on untreated and treated tomatoes were not significantly different (Table 3). Overall, reductions in yeast and mold populations on tomatoes treated with ClO_2 gas were less than those on treated apples, supporting the hypothesis that interactions between fungal propagules and the epidermis of tomatoes retard penetration of ClO_2 gas *(9)*.

Subjective evaluation revealed no apparent visual differences in overall appearance and color of tomatoes after treatment with 1.4, 2.7, or 4.1 mg/liter ClO_2 for 6, 12, and 25 min, respectively, at 34 to 62% relative humidity. Sensory quality ratings assigned to untreated and treated (2.7 mg/liter ClO_2) tomatoes stored at 21°C for up to 10 days are listed in Table 4. Treatment did not adversely affect quality. Treated tomatoes were rated significantly higher ($\alpha = 0.05$) for appearance on day 10, color on days 3, 7, and 10, aroma on day 10, and overall quality on days 7 and 10. Treatment with 2.7 mg/liter ClO_2 promoted the development of red color within 3 days. Decreased sensory quality ratings of treated and untreated tomatoes between 3 and 7 days and again between 7 and 10 days are attributed to overripening and decay caused by molds. The 2 log CFU per tomato reduction in *Salmonella* caused by treatment with 2.7 mg/liter ClO_2 (Table 3) and the lack of an adverse effect on sensory quality make this treatment promising in for commercial application.

Treatment of onions. Vidalia onions inoculated with *Salmonella* were treated with ClO_2 gas at concentrations of 1.4, 2.7, and 4.1 mg/liter within 5.4, 10.4, and 20 min,

TABLE 4. *Mean hedonic ratings for sensory attributes of produce treated with 1.4 mg/liter ClO_2 gas*

Produce	Storage time at 21°C (days)	Amount of ClO_2 released (mg/liter)	Ratings for sensory attributes[a]: Appearance	Color	Aroma	Overall quality
Apple	0	0	A 6.9 X	A 6.8 X	B 5.5 X	A 6.8 X
		1.4	A 6.7 Y	A 6.6 Y	C 5.4 X	A 6.5 Y
	9	0	A 6.8 X	A 6.8 X	B 5.7 X	A 6.7 X
		1.4	B 6.0 Y	B 6.0 Y	B 5.6 X	B 6.0 Y
	20	0	A 6.8 X	A 6.7 X	A 6.1 X	A 6.7 X
		1.4	B 6.2 Y	B 6.1 Y	A 5.9 Y	B 6.2 Y
	31	0	B 5.8 X	B 5.5 X	A 6.0 X	B 5.9 X
		1.4	C 5.3 Y	C 5.4 Y	A 5.9 X	C 5.4 Y
	41	0	C 5.4 X	C 5.5 X	A 5.9 X	C 5.5 X
		1.4	D 5.1 Y	C 5.2 Y	A 5.9 X	D 5.1 Y
Tomato	0	0	A 7.3 X	A 7.3 X	A 6.3 X	A 7.1 X
		2.7	A 7.3 X	A 7.3 X	B 5.6 X	A 6.9 Y
	3	0	A 7.1 X	A 7.0 Y	A 6.6 X	A 7.0 X
		2.7	A 7.2 X	A 7.3 X	A 6.4 X	A 7.1 X
	7	0	B 4.6 X	B 5.1 Y	B 3.6 X	B 4.1 X
		2.7	B 5.0 X	B 5.5 X	C 3.8 X	B 4.3 X
	10	0	C 1.9 Y	C 2.2 Y	C 1.8 Y	C 1.8 Y
		2.7	C 3.6 X	C 4.0 X	D 2.7 X	C 3.2 X
Onion	0	0	A 6.3 X	A 6.2 X	A 5.6 X	A 6.3 X
		4.1	A 6.2 X	A 6.2 X	A 5.2 Y	A 7.4 Y
	12	0	A 6.3 X	A 6.2 X	A 5.6 X	A 6.2 X
		4.1	B 5.4 Y	B 5.5 Y	A 5.1 Y	B 5.4 Y
	20	0	B 5.6 X	B 5.6 X	A 5.4 X	B 5.6 X
		4.1	C 5.0 Y	C 5.0 Y	AB 5.0 X	C 5.0 Y
	31	0	C 4.7 X	C 4.7 X	B 5.1 X	C 4.7 X
		4.1	D 4.4 X	D 4.5 X	B 4.7 X	D 4.5 X
Peach	0	0	B 7.2 X	B 7.1 X	C 6.5 X	B 7.0 X
		4.1	A 6.8 Y	A 6.4 Y	B 4.8 Y	A 6.4 Y
	3	0	A 7.6 X	A 7.8 X	A 7.9 X	A 7.7 X
		4.1	B 2.7 Y	B 2.6 Y	B 4.8 Y	B 2.8 Y
	7	0	C 6.5 X	BC 6.8 X	B 7.2 X	C 6.6 X
		4.1	C 1.6 Y	C 1.6 Y	C 4.2 Y	C 5.9 Y
	10	0	C 6.5 X	C 6.6 X	B 7.2 X	C 6.6 X
		4.1	D 1.2 Y	D 1.3 Y	D 2.7 Y	D 1.3 Y

[a] Ratings were assigned by panelists using a 9-point hedonic scale: 1 = dislike extremely, 5 = neither like nor dislike, and 9 = dislike extremely. Within the same produce type, treatment, and attribute, mean values not preceded by the same letter are significantly different ($\alpha = 0.05$). Within the same produce type, storage time, and attribute, mean values not followed by the same letter are significantly different ($\alpha = 0.05$).

respectively, at 22 ± 1°C and 35 to 64% relative humidity (Fig. 5). Significant reductions ($\alpha = 0.05$) in populations occurred compared with populations on untreated onions (Table 3). The highest treatment concentration (4.1 mg/liter ClO_2) caused the highest log reduction (1.94 log CFU per onion), although reductions resulting from all ClO_2 treatments were not significantly different. The log reductions in *Salmonella* populations achieved by treatment with ClO_2 gas were overall less than those observed for treated apples, tomatoes, or peaches. This reduced effect of the gas may have been caused by several factors. Onion skin is more porous and less uniform compared with the smooth and waxy surfaces of apples and tomatoes and thus may harbor more soil. The porous onion skin also may facilitate infiltration of *Salmonella* and other microorganisms, a condition observed in other produce *(9, 12, 38, 45)*. The concentric nature of onion layers may provide protection of *Salmonella* by preventing direct exposure to ClO_2 gas. The presence of soil, debris, and other organic and inorganic material may result in reduced effectiveness of ClO_2 gas, as has been observed with chlorine *(16)* and ozone *(31, 35)*.

The number of yeasts and molds recovered from untreated and treated onions was not significantly different (Table 3). Overall, reductions in populations were much smaller for onions than for apples, tomatoes, or peaches. The same phenomenon affecting survival of *Salmonella* on onions treated with ClO_2 also may have been partly responsible for survival of yeasts and molds. The higher initial population of yeasts and molds on onions compared with other produce, presence of different genera or species of yeasts and molds on the four types of produce, and the type, location, and number of mycelia, spores, and conidia may also be reflected in differences in reductions in counts resulting from ClO_2 treatment.

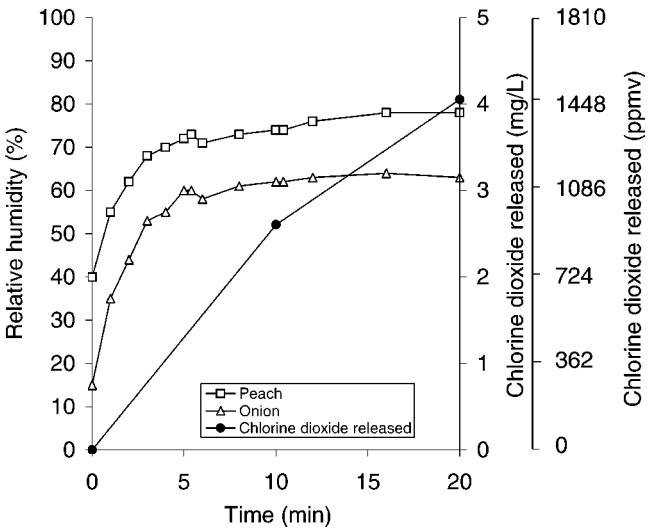

FIGURE 5. *Atmospheric relative humidity (□, △) in the cabinet housing whole onions and peaches, respectively, that were treated with ClO_2 gas (●) released into the cabinet.*

Immediately following treatment with 1.4, 2.7, or 4.1 mg/liter ClO_2 gas for 5.4, 10.4, and 20 min, respectively, at 36 to 64% relative humidity, onions were subjectively examined for changes in appearance and color. No differences were detected. Sensory qualities of onions treated with 4.1 mg/liter ClO_2 were slightly but significantly lower ($\alpha = 0.05$) than those of untreated onions after storage for 12 or 20 days at 21°C (Table 4). Significant differences in sensory attribute ratings for treated and untreated onions were not detected on day 31. A reduction in *Salmonella* by 1.9 log CFU per onion resulted from treatment with 2.7 mg/liter ClO_2 without greatly compromising sensory quality, indicating that ClO_2 gas treatment of onions should be investigated on a larger scale.

Treatment of peaches. Peaches inoculated with *Salmonella* were treated with ClO_2 gas at concentrations of 1.4, 2.7, and 4.1 mg/liter within 5.4, 10.4, and 20 min, respectively, at 21 ± 1°C and 55 to 78% relative humidity (Fig. 5). Significant reductions ($\alpha = 0.05$) compared with populations recovered from untreated peaches were observed (Table 3). The highest treatment concentration (4.1 mg/liter) caused the greatest log reduction (3.23 log CFU per peach), although populations recovered from peaches treated with all test concentrations of ClO_2 were not significantly different. Log reductions of *Salmonella* on treated peaches were slightly less than those on apples and tomatoes but greater than those on onions. These results were not unexpected because the open surface of broken trichomes on peaches would provide protected sites for *Salmonella* against contact with ClO_2 gas. The greater log reductions of *Salmonella* on treated peaches compared with reductions on treated onions may be attributed in part to the surface of peaches being less porous and containing less debris, soil, and other materials that may interact with ClO_2 gas and prevent contact with the pathogens. Yeast and mold populations on untreated peaches and peaches treated with 1.4 mg/liter ClO_2 were not significantly different, but populations recovered from peaches treated with 2.7 or 4.1 mg/liter ClO_2 were significantly lower than those recovered from untreated peaches. Overall, reduction of yeast and mold populations on peaches treated with ClO_2 was greater than those achieved by treatment of apples, tomatoes, and onions.

Immediately following treatment of peaches with 1.4, 2.7, and 4.1 mg/liter ClO_2 gas, changes in appearance and color were not evident by cursory examination. However, sensory evaluation of peaches treated with 4.1 mg/liter ClO_2 revealed that significant reductions in quality ($\alpha = 0.05$) were immediate, and deterioration rapidly progressed during storage at 21°C for 3, 7, and 10 days (Table 4). Although ratings for all sensory attributes of untreated peaches significantly increased during the first 3 days, which can be attributed to ripening, ratings for treated peaches significantly and dramatically decreased. Loss of sensory quality is attributed to extensive browning of treated peaches. Thus, although treatment of peaches with 4.1 mg/liter ClO_2 reduced the population of *Salmonella* by 3.23 log CFU per peach (Table 3), application at this concentration is not practical because sensory qualities are rapidly and markedly decreased.

ClO_2 gas shows promise as a sanitizer to substantially reduce populations of pathogens on fresh-cut cabbage, fresh-cut carrots, and whole apples, tomatoes, and peaches but may not be as useful for fresh-cut lettuce and whole onions. With the exceptions of apples, tomatoes, and onions, treatment with ClO_2 at concentrations high enough to reduce pathogen populations to numbers similar to those obtained using chlorine can adversely affect sensory quality of test fruits and vegetables. Identification of structures and specific components in produce tissues that may enhance the survival or promote inactivation of foodborne pathogens, yeasts, and molds should be further studied. The effects of a more rapid release of ClO_2 gas on inactivation of pathogens and on sensory quality should also be investigated.

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