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Chloroxyanion Residues in Cantaloupe and Tomatoes after Chlorine Dioxide Gas Sanitation

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Supporting Information

ABSTRACT: Chlorine dioxide gas is effective at cleansing fruits and vegetables of bacterial pathogens and(or) rot organisms, but little data are available on chemical residues remaining subsequent to chlorine gas treatment. Therefore, studies were conducted to quantify chlorate and perchlorate residues after tomato and cantaloupe treatment with chlorine dioxide gas. Treatments delivered 50 mg of chlorine dioxide gas per kg of tomato (2-h treatment) and 100 mg of gas per kg of cantaloupe (6-h treatment) in sealed, darkened containers. Chlorate residues in tomato and cantaloupe edible flesh homogenates were less than the LC−MS/MS limit of quantitation (60 and 30 ng/g respectively), but were 1319 ± 247 ng/g in rind + edible flesh of cantaloupe. Perchlorate residues in all fractions of chlorine dioxide-treated tomatoes and cantaloupe were not different ($P > 0.05$) than perchlorate residues in similar fractions of untreated tomatoes and cantaloupe. Data from this study suggest that chlorine dioxide sanitation of edible vegetables and melons can be conducted without the formation of unwanted residues in edible fractions.

KEYWORDS: *chlorine dioxide, chlorate, perchlorate, chlorite, residue, food safety*

INTRODUCTION

Chlorine dioxide (ClO_2) gas is a strong oxidizer that is highly effective at inactivating bacterial pathogens[1] and spores,[1] amoeba,[2] fungi,[3,4] rot organisms,[1] viruses,[5,6] and even insects.[7] In the United States, aqueous-based chlorine dioxide disinfectants and sanitizers have been approved by the US EPA for a diverse number of farm, bottling plant, and food processing, handling, and storage applications[8] including fruit and vegetable washes, flume water disinfection, meat and poultry treatment, food processing plant disinfection, water sanitation, odor control, medical waste disinfection, and municipal water treatment. Gaseous chlorine dioxide is approved as a sterilant for a variety of manufacturing and laboratory applications including the treatment of environmental surfaces, tools, and clean rooms. The gas is also used for odor control in a variety of settings. Chlorine dioxide gas has advantages over aqueous formulations because of its rapid diffusion, ease of mixing with air, and especially its ability to penetrate porous surfaces.[5,9]

Although efficacy of the gas against specific zoonotic and plant pathogens, including *Listeria monocytogenes*,[10,11] *E. coli* O157:H7,[12] and *Salmonella enterica*[13] is generally well-known, the use of gaseous chlorine dioxide on vegetables is not authorized by regulatory agencies. The major obstacle precluding regulatory approval for vegetable applications has been the lack of data describing chlorine dioxide's fate and chemical disposition on sanitized crop groups. To this end, Trinetta et al.[14] studied the fate of ClO_2 gas after surface application to tomatoes, oranges, apples, strawberries, lettuce, alfalfa sprouts, and cantaloupe using a colorimetric assay for ClO_2 and an ion chromatographic method for ions including chlorite, chlorate, and chloride. Whereas they concluded that "chlorine dioxide technology leaves minimal to no detectable chemical residues. . .", they did find extremely high concentrations of some chloroxyanions (chlorate on alfalfa sprouts exceeding 18 000 ppm, and neary 800 ppm chlorite on lettuce, for example) in water rinses collected the day of fumigation. Trinetta et al.[15] also used sufficiently high concentrations of gas during 10 min exposures to cause "significant discoloration, browning, and bleaching, due to gas treatment" on produce containing high concentrations of residue. Others[11,16,17] have also reported chlorite residues of greater than 1 mg/kg on strawberries and lettuce rinses subsequent to treatment with excess chlorine dioxide gas. An alternative approach to chlorine dioxide sanitation of produce involves longer duration (hours) treatment with fairly low chlorine dioxide gas concentrations using technology that provides a defined release of chlorine dioxide over time. Residues remaining on sanitized produce under mild treatment conditions have not been previously investigated.

Our laboratory has investigated the fate and disposition of radiolabeled chlorine dioxide gas ($^{36}ClO_2$) on tomatoes (50 mg/kg) and cantaloupe (100 mg/kg) during 2-h treatment periods.[18] The studies clearly indicated that radioactivity from $^{36}ClO_2$ (g) treatment was deposited on the surfaces of vegetable matter, especially on moist surfaces such as stem scars. The data also indicated that radioactive residues were not present in edible flesh of cantaloupe after $^{36}ClO_2$ treatment, but that ample residue was present on cantaloupe rind. Thus,

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Table 1. Temperature and Relative Humidity Measurements during Chlorine Dioxide Sanitation of Tomatoes and Cantaloupe

	tomato status			cantaloupe		
	present		absent	present		absent
time	(−) ClO_2	(+) ClO_2	(+) ClO_2[a]	(−) ClO_2	(+) ClO_2	(+) ClO_2
min	temperature °C					
0	20.3 ± 1.9	20.8 ± 1.0	23.5 ± 1.4	19.7 ± 1.5	15.7 ± 2.1	19.7 ± 1.2
30	19.0 ± 1.4	20.3 ± 0.9	23.4 ± 1.3			
60	18.9 ± 1.3	20.3 ± 0.9	23.4 ± 1.2	18.3 ± 1.2	16.0 ± 1.7	19.3 ± 0.6
90	19.0 ± 1.3	20.4 ± 0.8	23.4 ± 1.2			
120	19.2 ± 1.2	20.5 ± 0.8	23.3 ± 1.1	19.7 ± 0.6	16.7 ± 1.2	19.0 ± 0.0
240				20.7 ± 1.5	18.3 ± 1.5	19.3 ± 1.2
360				21.7 ± 1.5	20.0 ± 1.7	19.3 ± 1.2
	relative humidity (%)					
0	28 ± 1.2	33 ± 2.0	24 ± 0.7	42 ± 7.4	55 ± 8.7	37 ± 5.1
30	42 ± 1.0	44 ± 2.1	25 ± 0.0			
60	45 ± 1.5	47 ± 1.7	26 ± 1.4	64 ± 1.5	69 ± 0.6	48 ± 2.3
90	48 ± 1.5	50 ± 1.7	29 ± 0.7			
120	51 ± 2.1	53 ± 2.1	30 ± 0.0	69 ± 1.5	72 ± 1.0	49 ± 2.6
240				77 ± 2.0	78 ± 1.7	49 ± 2.6
360				84 ± 1.5	82 ± 1.2	49 ± 2.3

[a]Means of two replicates for the tomato study; the temperature and humidity were not measured on a single replicate of tomatoes.

gaseous sanitation with $^{36}ClO_2$ was a surface phenomenon. Kaur et al.,[19] using a higher specific activity label than Smith et al.,[18] generated essentially identical results: $^{36}ClO_2$ treatment resulted in substantial total radioactive residue on cantaloupe surface, but nondetectable radioactive residues in the edible flesh portion of the melon.

Also clearly established by tracer studies using tomatoes[18] and cantaloupe[18,19] is that the most prevalent chemical residue related to $^{36}ClO_2$ (g) exposure was chloride ion (Cl^-), a ubiquitous nutrient which from a food-safety standpoint can be ignored. Chloride is the five electron reduction product of ClO_2 gas. Under certain conditions, however, two chloroxyanion byproducts, chlorate[18,19] (ClO_3^-) and perchlorate[18] (ClO_4^-) may also form during chlorine dioxide sanitation. The formation of chlorate and perchlorate can be minimized or essentially eliminated, however, if chlorine dioxide sanitation processes are protected from light.[18] In contrast to previous reports[11,14,16] one chloroxyanion byproduct that was not measured in either cantaloupe or tomatoes after $^{36}ClO_2$ (g) treatment was chlorite[18,19] (ClO_2^-). In fact, ^{36}Cl-labeled chlorite ion specifically fortified into tomatoes was quantitatively transformed to chloride and chlorate ions.[18]

Collectively, efficacy and chemical residue data suggest that the use of chlorine dioxide gas could be a highly effective, yet safe, tool for pathogen or rot organism reduction on vegetable matter. However, chemical residues of ClO_2-treated vegetable matter have not been assessed in experiments other than laboratory-scale, single exposure experiments using radiolabeled tracer materials. The purpose of this study, therefore, was to determine the magnitude of chlorate and perchlorate residues on kg-scale quantities of tomatoes and cantaloupe after sanitation with a slow-release chlorine dioxide formulation.

MATERIALS AND METHODS

Chlorine Dioxide Generation. Chlorine dioxide gas [ClO_2; CAS 10049−04−04] generation was effected using a two-part dry media system (ICA TriNova; Atlanta, GA) consisting of a zeolite carrier impregnated with sodium chlorite (Dry Media A) and an acid activator ($FeCl_3$; Part B) in a proprietary formulation. After parts A and B are mixed, chlorine dioxide gas is released in a predictable and repeatable manner.[13,20,21]

Tomato Experiments. *Containers and Accessories.* Polyethylene food storage tubs (46 × 66 × 38 cm, W × D × H; 83 L; Cambro, Huntington Beach, CA) and lids were prepared to accommodate individual flats of tomatoes, two 13 cm fans (O2Cool; Chicago, IL), a remote humidity/temperature detector (no. 14-649-84; Fisher Scientific, Pittsburgh, PA), and the gas generating media. Gas sampling ports (12 mm hole approximately 12 cm below the tub rim) were added and sealed with a butyl stopper (no. 73828A-21; Kimble Chase, Vineland, NJ) and silicone sealant. Just prior to the initiation of each experiment, container lids were lined with a thick bead of 100% silicone rubber sealant (no. 8648; DAP, Baltimore, MD).

Tomato Treatment. Ripened Beefsteak tomatoes (approximately 300 g each; DiCiocco Farms, Ontario Canada) were stored in a walk-in cooler (4−5 °C) until use. Tomatoes were removed from the cooler 1 to 1.5 h prior to the initiation an experiment and weighed to the nearest g (Table 1). Two fans were placed on the floor of each chamber and were turned on; the temperature/humidity probe was placed into the chamber. Flats containing tomatoes (n = 22 per experiment; approximately 6.6 kg total) were placed approximately 15 cm above the floor of the chamber, above the fans, on polyethylene racks. Treatments were initiated by mixing 45 g each of ICA TriNova (Newnan, GA) dry media parts A and B within a Tyvek sachet, agitating the sachet by hand to facilitate mixing, and placing the sealed sachet into the treatment chamber, but not onto the tomatoes. Sufficient media was provided to generate a target of 50 mg of chlorine dioxide per kg of tomato during a 2 h treatment period. Lids were sealed securely on each reaction chamber. Temperature and percentage relative humidity were recorded at 0, 30, 60, 90, and 120 min by reading values directly from the temperature/humidity meter. Chamber gases (5−10 mL) were removed from gas sampling ports at 0, 5, 10, 20, 30, 45, 60, 90, and 120 min using a 10 mL gastight syringe (SGE Analytical; 008960) equipped with a 19-ga syringe needle and assayed immediately for ClO_2 gas as described below.

A single chlorine dioxide treatment chamber and a single control chamber were run each d for 3 consecutive d. A third set of chambers was set up to monitor chlorine dioxide production in the absence of tomatoes. All chlorine dioxide treatments were protected from light by turning the laboratory lights off; light intensity during the sanitation process was 4−5 lx.

At the termination of the 2-h sanitation period, lids were removed from treatment tanks, tomato flats were removed, and sachets containing the ClO_2 generating media were discarded. Triplicate sets

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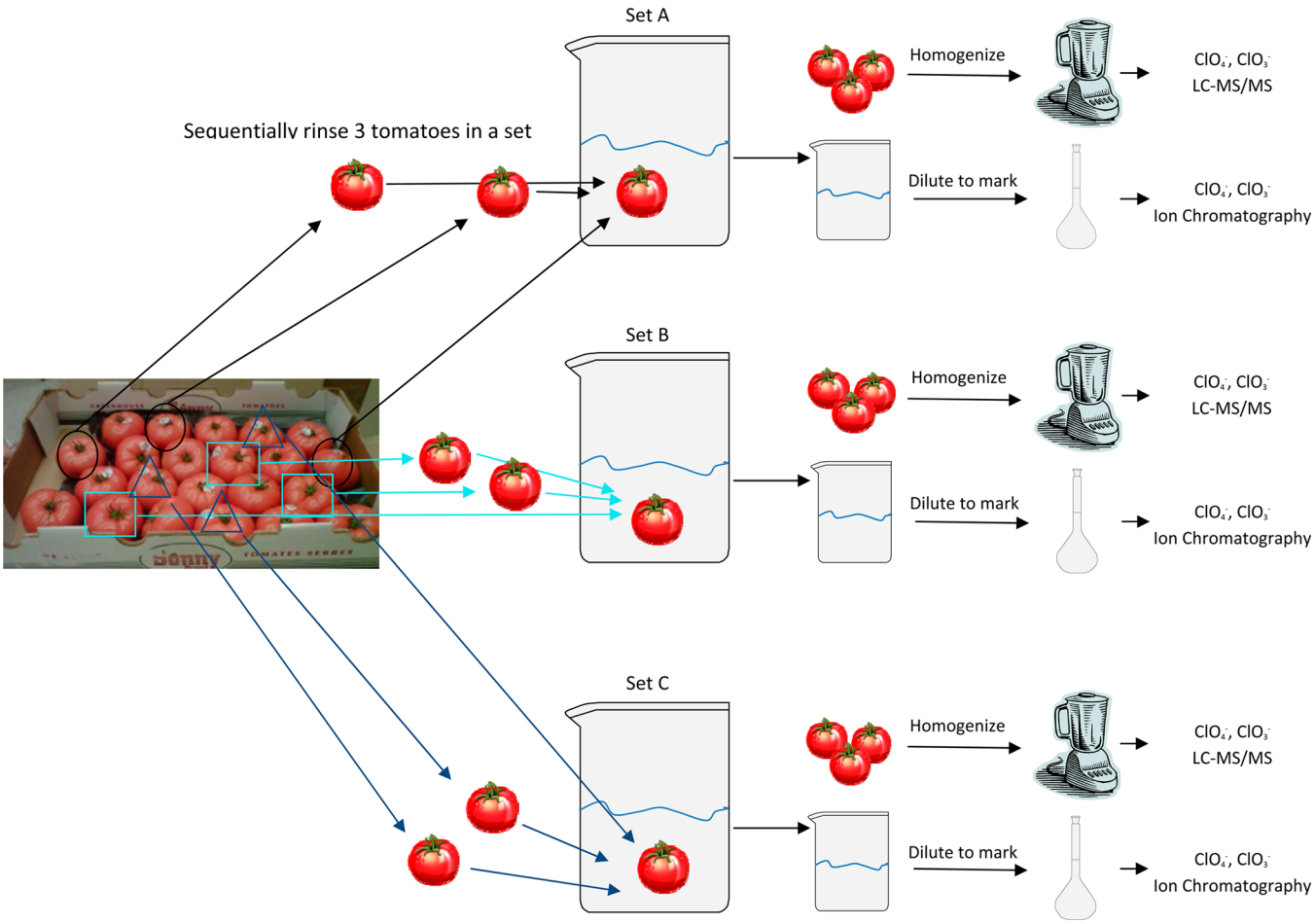

Figure 1. Schematic showing the handling of within-day tomato subsamples (Sets A, B, and C), the within set sequential rinsing of individual tomatoes, and processing of tomato sets and water rinses.

of 3 tomatoes each were removed from each flat for further processing (Figure 1). Tomato sets were weighed, and individual tomatoes within each set were sequentially rinsed in 400 mL of water (>18 MΩ cm) contained within a respective 1-L beaker (Figure 1). Rinse water for each tomato set was transferred to a 500 mL volumetric flask, diluted to the mark with purified water (>18 MΩ cm), and mixed thoroughly by inversion. Aliquots (50 mL) of each rinse fraction were placed into labeled containers and frozen (−20 °C or less) until analyses. Tomato rinses were analyzed for chlorate and perchlorate as described below.

Tomato Processing. Tomato sets were pureed in a food processor. Four 50 mL portions of the puree from each set were placed into 50 mL tubes, capped, and frozen (−20 °C or less) until analysis. Tomatoes were analyzed for perchlorate and chlorate content as described below.

Cantaloupe Experiments. *Containers and Accessories.* Polyethylene storage tubs; Rubbermaid Roughneck #3AO5; 85 × 41 × 43 cm, L × W × H; 208 L) and lids were each prepared to accommodate two cartons of cantaloupe (12 cantaloupe per carton), two 13 cm fans (O2Cool; Chicago, IL), a remote humidity/temperature detector and a Tyvek sachet as described for tomatoes. Gas sampling ports, butyl stopper seals, and silicone rubber sealant were added to containers as described for the tomato experiments.

Cantaloupe Treatment. Twenty-four cantaloupe (12-Count; Del Monte #4050, Costa Rica), equally distributed in two cardboard crates, were weighed and placed into tubs. Chlorine dioxide treatment was initiated by mixing 314 to 328 g each of dry media parts A and B within a Tyvek sachet, agitating the sachet by hand to facilitate mixing, and placing the sealed sachet into the treatment chamber (Figure 2). Sufficient media was provided to generate a target of 100 mg of chlorine dioxide per kg of cantaloupe during a 6-h treatment period. A third set of chambers (positive controls) was set up to monitor chlorine dioxide production in the absence of cantaloupe. All treatment chambers were protected from exposure to light by turning the laboratory lights off; light intensity during the sanitation process was 4−5 lx. Temperature and percentage relative humidity were recorded at 0, 60, 120, 240, and 360 min and chlorine dioxide concentration in 3 to 10 mL of chamber gas was measured at 0, 15, 30, 45, 60, 90, 120, 240, and 360 min as described for the tomato experiments. The experiment was replicated 3 times on each of 3 separate days; individual cantaloupes within a replicate were considered aliquots for which within-day residue means were calculated. Control cantaloupe treatments, which were not exposed to chlorine dioxide, consisted of a single crate of 12 melons.

Cantaloupe Processing. At the termination of the 6-h treatments, lids were removed from treatment tanks and the sachets containing ClO_2 generating media were discarded. Six cantaloupe (25% of total) were removed from treatment tanks and three cantaloupe (25% of total) were removed from control tanks with equal sampling from top and bottom layers of cantaloupe. Selected cantaloupes were weighed, bisected with a sharp knife, and the seed bed from both halves of each melon were removed, transferred to labeled containers, and weighed. The edible flesh was removed from one-half of each melon using a spoon and placed directly into a blender (Cuisinart CBT-500 or BFP-10CH, Stamford CT; or Oster BCCG08, Boca Raton, FL) where it was homogenized. Quintuplicate aliquots (~40 mL) of edible flesh homogenate from each melon were transferred to 50 mL polypropylene tubes (Sarstedt; Newton, NC; no. 62.554.002) and frozen (−20 °C). The remaining half of each melon was cut into strips and the rind with edible flesh was homogenized together.

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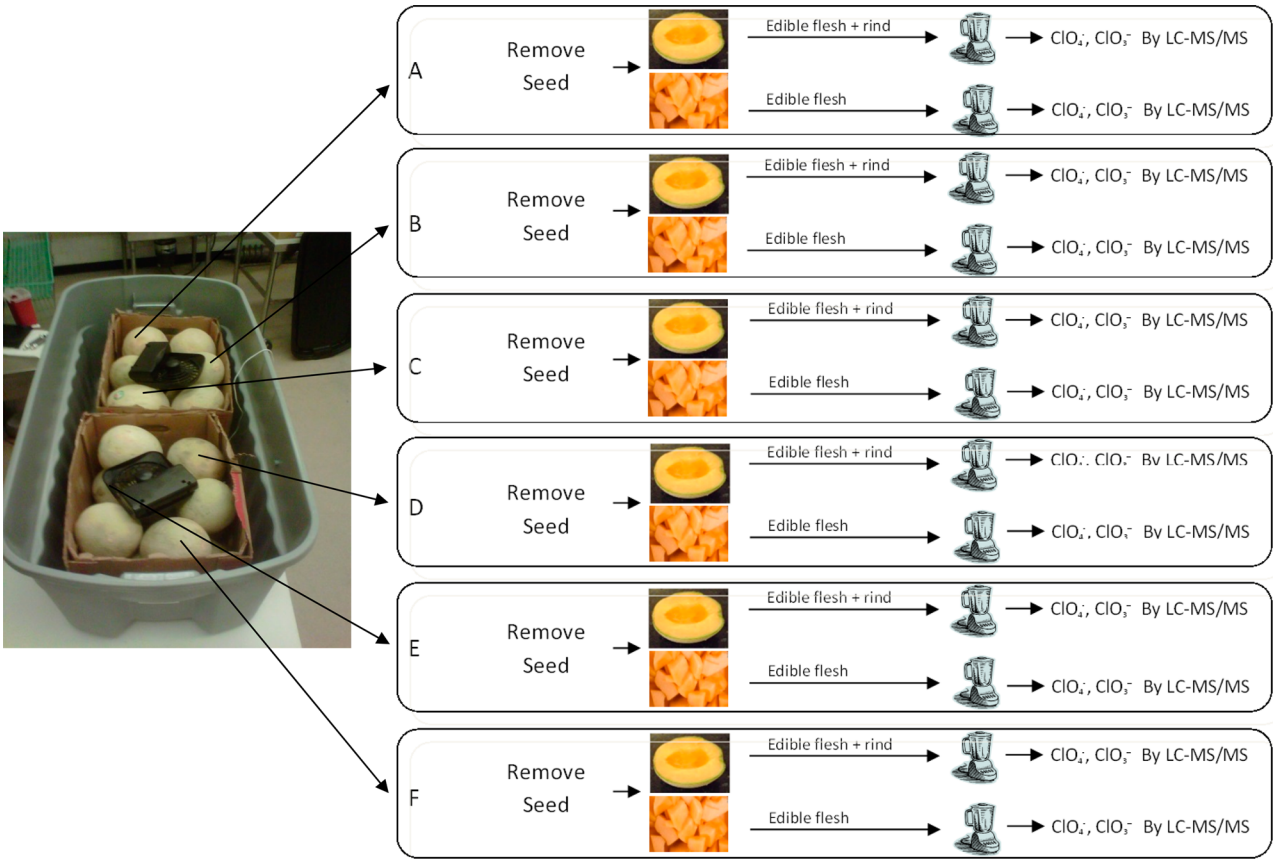

Figure 2. Daily processing of fumigated cantaloupe after ClO_2 sanitation. Of 24 cantaloupe (12 per crate; 2 crates) within an exposure tank, 3 melons were randomly selected from each crate for further processing and analysis. Equal numbers of cantaloupe were selected from the bottom and top melon layers. Cantaloupe were partitioned into edible flesh, edible flesh with rind, and seed bed fractions. Chlorate and perchlorate analyses were conducted on tank rinse, edible flesh, and edible flesh with rind fractions. Control tanks contained 12 melons, from which 3 were selected for further processing.

Quintuplicate aliquots (~40 mL) of rind and edible flesh homogenate were transferred to 50 mL polypropylene tubes and frozen.

Tank Rinse. Treatment tubs and lids were thoroughly rinsed by spraying all surfaces with nanopure (>18 MΩ cm) water from a spray container. Sequential rinses of a given tub were transferred, and pooled, into 2-L volumetric flasks. Upon completion of tank rinses, volumetric flasks were diluted to the mark, mixed thoroughly, and quintuplicate 40−45 mL aliquots were transferred into 50 mL polypropylene tubes and frozen (<−20 °C).

Chemical Analyses. *Chlorine Dioxide Assay.* A chlorine dioxide standard solution was prepared by reacting sodium chlorite with sulfuric acid as described by Ray et al.[22] Chlorine dioxide was trapped in ice-cold water after passing through a sodium chlorite column to remove Cl_2. The concentration of chlorine dioxide in the stock solution was determined by UV absorption (360 nm) of 1:10, 1:20, 1:50, and 1:100 dilutions of the stock solution in water. A molar absorption coefficient of 1225 M^{-1} cm^{-1} reported by Emmert et al.[23] was used to calculate chlorine dioxide concentration according to Beer's Law. The stock solution (0.738 ± 0.022 mg/mL) was stored sealed within a low actinic glass reservoir at 4 °C.

Chlorine dioxide concentrations in treatment chambers were measured using a Rhodamine-B based spectrophotometric assay as described by Xin and Jinyu.[24] Briefly, a standard curve containing concentrations of 0.1, 0.25, 0.5, 1.0, and 1.5 mg/L of chlorine dioxide was prepared by combining 2 mL of 10 mg/L rhodamine B, 2 mL of 1 M ammonia buffer (pH 10), and 2 mL of the an appropriate ClO_2 dilution into 25 mL volumetric flasks and diluting to the mark with purified water. After mixing, the absorbance of each vial was read at 553 nm.

Aliquots (5 to 10 mL) of gaseous chlorine dioxide were removed from containers at the indicated sampling times, bubbled immediately through respective mixtures of 1 mL of rhodamine B (10 mg L^{-1}), 1 mL of 1 M NH_3−NH_4Cl buffer (pH 10), and 10.5 mL nanopure water contained within individual 20 mL glass vials; absorbance (553 nm) was then measured using a Shimadzu (Kyoto, Japan) UV-1601 spectrophotometer. For the cantaloupe experiments, the total volume of the rhodamine B trapping solution was 25 mL, but with reagents combined in the same proportion. Concentrations of chlorine dioxide were determined using a standard curve prepared from standardized chlorine dioxide. Limits of quantitation for the Rhodamine-B chlorine dioxide assay were defined as the mean background concentration of chlorine dioxide in untreated tomato and cantaloupe tanks times 3 standard deviations of the mean.

Chlorate and Perchlorate in Rinse Waters. Perchlorate analyses of tomato rinses and cantaloupe tank rinse waters were conducted using a Thermo-Fisher ICS-2100 ion chromatograph using the framework outlined in EPA method 314.0.[25] Sample aliquots (1 mL) were injected onto a Dionex AS16 column (4 mm × 250 mm) protected by an AG16 guard column (4 mm × 50 mm). An isocratic mobile phase of 50 mM KOH, prepared using a Thermo-Fisher eluent generator, with a flow rate of 1.5 mL/min was used to elute perchlorate from the column. Perchlorate was measured using suppressed conductivity detection (Thermo-Fisher DS6) in-line with eluent-recycled (ASRS 300) suppression (186 mA). Sodium perchlorate standards (1, 5, 10, 50, 100, and 200 μg/L), prepared in nanopure water (>18 MΩ cm), were injected in replicate 1 mL aliquots onto the ion chromatograph with each sample set. Peak areas associated with perchlorate standards were regressed against perchlorate concentration using Chromeleon CHM-2 software. The least-squared regression equations were then

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used to predict the concentration of perchlorate in the experimentally obtained samples. The method detection limit (MDL) was calculated as follows:

$$\mathrm{MDL} = (t)x(S_{n-1})$$

where t is the student's t value for a 99% confidence interval (3.14 for seven replicates), and S_{n-1} is the sample standard deviation $(n-1)$ for seven replicates of the 5 ppb perchlorate standard. The MDL for perchlorate was measured contemporaneously with sample sets.

Chlorate analyses of tomato rinses and cantaloupe tank rinse waters were conducted using the same chromatograph as used for the perchlorate analyses. Standards consisting of 1, 5, 10, 50, 100, and 200 μg/L of sodium chlorate were prepared in nanopure water. Chlorate was separated from interferences on a 4 mm × 250 mm Dionex AS19 column protected by a 4 mm × 50 mm AG19 guard column with an isocratic mobile phase of 20 mM KOH flowing at 1.0 mL/min. Mobile phase was prepared using a Thermo Fisher eluent generator. Ions were detected using a DS6 conductivity detector with recycled-eluent suppression (ASRS 300; 50 mA). Sodium chlorate standards were run at the beginning and end of each sample set. Blank samples were also concurrently run with each analysis. Concentrations of chlorate in unknowns were determined using least-squared regression of peak areas of known standards. Method limits of detection were determined as described for perchlorate using the 5 ppb chlorate standard.

Perchlorate Residues in Tomato and Cantaloupe. Perchlorate in tomato puree was analyzed using the Krynitsky et al.[26] method employed by the US-Food and Drug Administration during their 2004−2005 survey of perchlorate in food.[27] Briefly, 10-g aliquots of thawed tomato puree were weighed into 50 mL conical tubes in duplicate. Sample sets included fortified control (30 ng/g) tomatoes, chlorine dioxide fumigated tomatoes, and control tomatoes. Fortified control tomatoes were obtained from a local source and were previously determined to be perchlorate free. All samples were fortified with 30 ng/g of ^{18}O-labeled perchlorate internal standard (Icon Services, Inc.; Summit, NJ). Each tube was diluted with 20 mL of 1% acetic acid and mixed at high speed for 2 min on a Rotamix (ATR; Laurel, MD) and subsequently centrifuged at 30 600 × g for 15 min on a Sorvall centrifuge at 4 °C. Supernatants were decanted into 50 mL tubes and placed on ice. Aliquots (6.5 mL) of supernatant were subsequently loaded onto preconditioned (6 mL acetonitrile followed by 6 mL of 1% acetic acid) ENVI-Carb (500 mg, 6 cc) solid phase extraction tubes. Perchlorate was not retained on the SPE tubes and was collected into tubes with the liquid portion of the tomato extract. Aliquots (1 mL) were subsequently filtered (0.2 μm PTFE filters) into 2 mL autosampler vials and 20 μL aliquots were analyzed by LC−MS/MS as described below.

Perchlorate residues were quantified in cantaloupe edible flesh and in rind with edible flesh exactly as described for tomatoes except that control cantaloupe was fortified with 150 ng/g of perchlorate; cantaloupe edible flesh was centrifuged at 30 600 × g and rind with edible flesh was centrifuged at 48 800 × g for 15 min.

Mass Spectrometry-Perchlorate. A Waters (Milford, MA) Acquity UPLC system online with a Waters triple-quadrupole mass selective detector was used to quantify perchlorate in tomato and cantaloupe edible flesh and edible flesh with rind extracts. Data were acquired, processed, and quantified using MassLynx 4.1 with QuanLynx software. Ion chromatograms were constructed for the ^{35}Cl transition m/z 99 → 83 for native perchlorate and the ^{35}Cl transition m/z 107 → 89 for ^{18}O-perchlorate. ^{37}Cl-Isotope transitions of native perchlorate were used for confirmatory purposes.[26] Sample aliquots (20 μL for tomato; 22 μL for cantaloupe) were injected from an autosampler maintained at 4 °C onto a Waters Ion-Pak Anion HR column (4.6 mm× 75 mm) maintained at 35 °C and eluted with an isocratic mobile phase of 100 mM ammonium acetate in 50% acetonitrile at a flow rate of 0.35 mL/min. Ions were detected in the negative ion mode with a capillary setting of 3.00 kV and a cone voltage of 65 V for $Cl^{18}O_4^-$ and ClO_4^-; the source and desolvation temperatures were set at 150 and 400 °C, respectively, with cone and desolvation gas flows at 50 and 800 L/h, respectively.

Calibration standards in water contained 0.25, 0.5, 1.0, 2.0, 5.0, 10.0, and 100 ng/mL of sodium perchlorate containing 10 ng/mL of internal sodium ^{18}O-perchlorate standard. Standard curves bracketed sample sets composed of fortified blanks, test samples, and control samples. The instrument limit of quantitation was 0.5 ng/mL with the method LOQ being 1.5 ng/mL after accounting for sample mass and dilution.[26] The instrument detection limit (0.08 ng/mL for tomato experiment, 0.10 ng/mL for cantaloupe experiment) was calculated as described above using the 0.5 ng/mL standard and a t value associated with (S_{n-1}) where S is the standard deviation associated with 12 observations. Accounting for dilution and sample mass, the method LOD was 0.24 ng/mL for the tomato experiment and 0.3 ng/mL for the cantaloupe experiments.

Mass Spectrometry-Chlorate. Relative to perchlorate, tomato puree and cantaloupe edible flesh and edible flesh with rind homogenates proved to be difficult matrices for the quantitation of chlorate anions by mass spectrometry. Matrix interferences prevented the use of an ^{18}O-labeled chlorate internal standard, and the response of the ^{37}Cl-isotope transition of m/z 84.7 → 68.7 was not linear with respect to concentration in fortified samples. Therefore, quantitation of chlorate in tomato matrix was based on the ^{35}Cl-isotope transition of m/z 82.7 → 66.7 using a matrix-matched standard curve. Briefly, aliquots (10 g) of puree or homogenate were prepared for mass spectral analysis exactly as described for perchlorate except that samples were not fortified with internal standard and fortified recovery samples were spiked with 120 ng/g of sodium chlorate. A matrix-matched calibration curve, prepared in the appropriate blank sample matrix, consisted of points at 10, 20, 40, 60, 80, and 100 pg/μL. For tomatoes, the 10 pg/μL matrix-matched standard did not routinely provide a signal-to-noise ratio greater than 5, so the limit of quantitation corresponded to the 20 pg/μL matrix-matched standard with the detection limit calculated as described for perchlorate in water. A corresponding method LOQ of 60 ng/mL resulted when sample mass and dilution were accounted for; the method limit of detection corresponded to 36 ng/g. For the cantaloupe extracts, the limit of detection for the instrument, as calculated from the 10 pg/μL sodium chlorate standard was 5 ng/μL. When sample mass and dilution were accounted for, the method LOD was 15 ng/g with a corresponding LOQ of 30 ng/g.

The Acquity UPLC system equipped with a Waters triple-quadrupole mass selective detector used to quantify perchlorate was also used to quantify chlorate in tomato extracts. Ion chromatograms were constructed for the ^{35}Cl-isotope transition of chlorate ion (m/z 82.7 → 66.7). Sample aliquots (22 μL) were injected from an autosampler maintained at 4 °C onto a Waters Ion-Pak Anion HR column (4.6 mm × 75 mm) maintained at 35 °C and eluted with an isocratic mobile phase of 100 mM ammonium acetate and acetonitrile (1:1) at a flow rate of 0.35 mL/min. Ions were detected in the negative ion mode with a capillary setting of 2.6 kV and a cone voltage of 45 V for ClO_3^-; the source and desolvation temperatures were set at 150 and 400 °C, respectively, with cone and desolvation gas flows at 50 and 800 L/h, respectively.

Because chlorate concentrations of cantaloupe rind with edible flesh exceeded the highest point of the calibration curve during the initial analysis, sample dilutions were required. For those samples, dilutions (1/4 to 1/10) were made by pipetting 1 part of sample extract into the appropriate amount of 1% acetic acid to a total volume of 1200 μL. Samples were then vortexed and analyzed by LC−MS/MS as described above. For example, a 1/4 dilution was performed by adding 300 μL of sample extract to 900 μL of 1% acetic acid; a 1/10 dilution was performed by adding 120 μL of sample extract to 1080 μL of 1% acetic acid.

Statistics. Differences in overall mean reaction tank temperatures were determined by a simple one-way ANOVA after pooling all temperature measurements across time within treatments. Bonferonni's multiple comparison test was used to infer differences in treatment means after the one-way ANOVA implied significant differences in means could have occurred. Effects of treatment on perchlorate and chlorate concentrations were determined by one-way ANOVA (SigmaPlot, 12.0) with significance set at $P < 0.05$.

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RESULTS AND DISCUSSION

Table 1 shows the temperatures and relative humidity during the tomato and cantaloupe treatments. Reactions were completed at temperatures which ranged from 15.7 to 23.5 °C. The lowest temperatures occurred in chambers containing cantaloupes which had been previously refrigerated, and the highest temperatures occurred in control tomato chambers in which no fruit were added. Relative humidity tended to increase in chambers containing tomatoes and cantaloupe as a function of time, but remained fairly consistent in control tanks over the incubation periods.

Figure 3 shows the theoretical release of ClO_2 gas into treatment chambers and shows the measured concentrations of

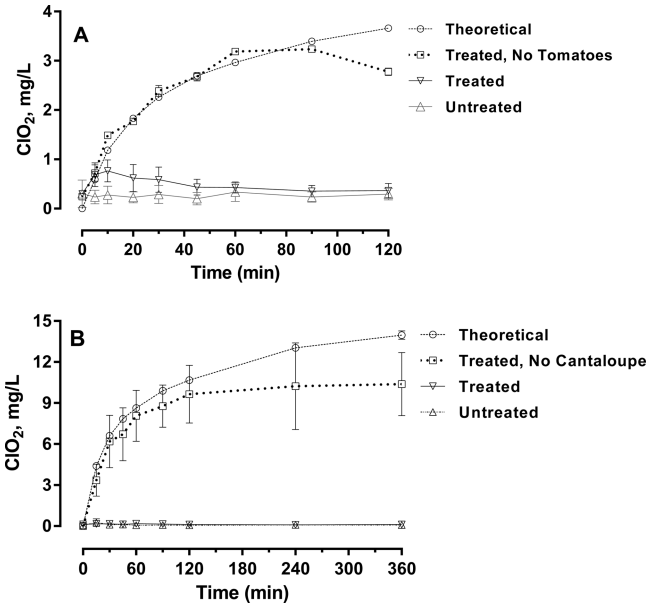

Figure 3. Mean chlorine dioxide concentrations (±standard deviations; n = 3 observations per treatment/time) in treatment tanks containing tomatoes (Panel A) or cantaloupe (Panel B). Data represent chlorine dioxide release in the absence of fruit (open squares), chlorine dioxide with fruit present (downward triangles), and in control tanks with no chlorine dioxide (upward triangles). Also shown is the theoretical release (open circles) of chlorine dioxide.

ClO_2 in control (no fruit) and fruit-laden chambers during sanitation. For both the tomato and cantaloupe experiments, chambers with an intact ClO_2 generating system, but without fruit, had ClO_2 concentrations that approximated theoretical values calculated based on ClO_2 release rates (provided by ICA TriNova; Newnan, GA) and chamber volumes. In the empty tomato chambers (Figure 3, panel A), gas concentrations approached 3 mg/L, whereas in the empty cantaloupe chambers (Figure 3, panel B) ClO_2 concentrations reached approximately 9 mg/L at 2 h and then remained relatively constant. In contrast, ClO_2 concentrations in treated tanks containing cantaloupe or tomatoes never approached theoretical levels, especially for cantaloupe (Figure 3, panel B). These data are highly consistent with studies employing $^{36}ClO_2$ that demonstrated the capacity of tomatoes and cantaloupe to adsorb ClO_2.[18,19] For instance, essentially all (>99.99%) of the radioactive residue associated with cantaloupe was on the inedible rind fraction of the melon.[18] In tomatoes, radioactive residues were highly concentrated in porous surfaces such as the stem scar where water exchange may take place.[18] Arango et al.[28] also demonstrated the capacity of produce to serve as a chlorine dioxide sink, establishing that strawberries consumed 15% of a 5 mg/L chlorine dioxide treatment within 7 min, and that chlorine dioxide absorption is a rapid, first-order process.

Since ClO_2 did not accumulate in tanks containing tomatoes or cantaloupe, a reasonable question is whether sufficient concentrations of gas for efficacy against pathogens and(or) rot organisms would be present when slow-release formulations are used. Previous researchers, however, have used similar slow release formulations to consistently reduce (>3 log units) *Salmonella*, *E. coli,* and(or) *Listeria* on the surfaces of apples, blueberries cabbage, carrots, lettuce, peaches, and tomatoes.[13,21,29−32] In addition, slow-release materials have also demonstrated efficacy against *Salmonella* on porous surfaces such as stem scars and surface wounds.[33] Further, rot or spoilage organisms including *Pseudomonas aeruginosa* and *Alicyclobacillus acidoterrestris* (spores) were reduced 5-log units or more on potatoes and apples, respectively, using slow-release ClO_2 materials.[34,35] Thus, the concentration of ClO_2 gas may not be as important as the total mass of gas delivered to fruit surfaces colonized by pathogen, rot, or spoilage organisms. An additional safety benefit with the slow release and rapid absorption of chlorine dioxide is that gas does not accumulate during sanitation. Because chlorine dioxide is a hazardous gas with implications for occupational exposures, the practical implications of nonaccumulating gas concentrations for sanitation facility infrastructure requirements and worker safety are obvious.

To be sure, gas concentrations in the empty tanks were sufficient to equal or surpass target concentrations of ClO_2 previously demonstrated to reduce *Escherichia coli* 0157:H7, *Salmonella,* and(or) *Listeria monocytogenes* on tomatoes[13,33,36] and cantaloupe[37] by 3.5 to 5 log units. Gas concentrations in the cantaloupe experiment did not reach the 10 mg/L concentration used by Trinetta et al.[15] to demonstrate the very rapid (180 s) inactivation of pathogens on tomatoes, cantaloupe, and strawberries.

Studies published after tomato or cantaloupe treatment with $^{36}ClO_2$ have established that chlorate (ClO_3^-) and chloride (Cl^-) are the major residues formed during ClO_2 sanitation of tomatoes and cantaloupe;[18,19] formation of perchlorate (ClO_4^-) may occur under sanitation conditions[18] of high gas concentration and exposure to light. Because perchlorate and chlorate are the stable residues formed, we investigated their presence in rinses of tomatoes and reaction chambers and on tomatoes and cantaloupe. Table 2 shows perchlorate and chlorate residues present in tomato rinsewater and in cantaloupe tank rinsewater from this study. Detectable (>1.3 ng/mL) perchlorate was not present in any of the tomato rinse fractions, nor was perchlorate present in the tank rinse samples of the cantaloupe experiments. The absence of perchlorate in rinses of chambers containing either tomatoes or cantaloupe is not surprising since the vegetable matter acted as chlorine dioxide sinks (Figure 3) which prevented the accumulation of chlorine dioxide gas. The fact that chlorine dioxide did not accumulate in reaction chambers likely contributed to the fact that no perchlorate was detected in rinse fractions. However, a more important factor was the absence of light during the sanitation process. For example, the absence of perchlorate in control tank rinses of the cantaloupe experiment (where chlorine dioxide did accumulate) demonstrated that in the absence of a light catalyst, the formation of perchlorate residues was prevented completely. The light-catalyzed degradation[38]

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Table 2. Concentrations of Chlorate and Perchlorate in Water Rinses of Tomatoes, And in Water Rinses of Sanitation Chambers after Treatment of Cantaloupe with Chlorine Dioxide Gas[a]

treatment	chlorate (ng/mL)	perchlorate (ng/mL)
tomatoes		
control, no ClO_2	<LOD[b]	<LOD[b]
treated, ClO_2 (+) tomatoes	6.8 ± 1.9[c]	<LOD
cantaloupe		
control, no ClO_2	<LOD[d]	<LOD[d]
treated, ClO_2 (+) cantaloupe	(4.3 ± 1.6)[e]	<LOD[d]
treated, ClO_2 (−) cantaloupe	82.1 ± 31.9	<LOD[d]

[a]Values represent means ± standard deviations of three sanitation experiments each with tomatoes and cantaloupe. [b]LOD, limit of detection for chlorate and perchlorate in tomato rinsewater was 1 and 1.3 ng/mL, respectively. [c]Two of three replicates had residues above the limit of quantitation (5.0 ng/mL); a single replicate had residues at the limit of quantitation. The mean was calculated by including the values of the single replicate having a chlorate concentration at the LOQ. [d]LOD, limit of detection for chlorate and perchlorate in cantaloupe tank rinsewater was 3.3 and 1.7 ng/mL, respectively. [e]Two of three replicates had residues below the limit of quantitation (5.0 ng/mL) but above the limit of detection (3.3 ng/mL), while one replicate had residues above the limit of quantitation. The mean was calculated by including the nominal values of the replicates having concentrations below the LOQ.

and oxidative formation of chlorate and(or) perchlorate from high concentrations of chlorine dioxide in gaseous[18,39,40] or aqueous phases[41−43] has been established in the absence of a chlorine dioxide sink as has the relative stability of gaseous chlorine dioxide gas when protected from light.[38] Previous radio labeled studies[18] surprisingly showed that even low gas concentrations in the presence of a sink could participate in light-catalyzed degradation, as small amounts of perchlorate and greater amounts of chlorate were detected when the experimental systems were exposed to light. Results from this study confirm the expectations from previous work that in the presence of a chlorine dioxide sink and in the absence of light, perchlorate formation from chlorine dioxide is nil.

Unlike perchlorate residues, chlorate residues were present in tomato rinsewater and cantaloupe tank rinsewater, albeit in low quantities (Table 2). As expected, chlorate was not present in rinses of negative controls, neither being in tomatoes rinses or tank rinses not exposed to chlorine dioxide gas. After chlorine dioxide treatment in the presence of fruit, however, low concentrations of chlorate were present in tomato rinsewater (6.8 ± 1.9 ng/mL) and in cantaloupe tank rinsewater (~4.3 ± 1.6 ng/mL). Chlorate rinsed from tomato surfaces or in rinses of cantaloupe tank chambers were just above (tomato rinses) and just below (cantaloupe tank rinses) the assay limit of quantitation (5 ng/mL). Rinse water from tanks containing ClO_2, but no cantaloupe, contained 82.1 ng/mL of chlorate. In the absence of a ClO_2 sink (cantaloupe), small quantities of chlorate were formed from ClO_2, very likely through disproportionation.[44]

Chlorate and perchlorate residues in tomato and cantaloupe homogenates are shown in Table 3. Recoveries of chlorate from blank samples fortified at 60, 120, 180, and 300 ng/g were 71.7 ± 7.8 (n = 5), 78.0 ± 8.9 (n = 6); 84.2 ± 3.8 (n = 5), and 78.4 ± 4.0%, respectively. Due to matrix interferences, the chlorate assay had an LOQ of 60 ng/g of tomato puree, and an LOD of 36 ng/g, which was substantially greater than the LOQ of 1.5

Table 3. Chlorate and Perchlorate Residues (ng/g) in Tomato Puree, Cantaloupe Edible Flesh, And Cantaloupe Edible Flesh + Rind after Treatment with Chlorine Dioxide[a]

treatment	chlorate (ng/g)	perchlorate (ng/g)
tomato puree		
control, no ClO_2	(52.2)[b]	8.8 ± 0.6
treated, ClO_2 (+) tomatoes	(45.1)[b]	9.3 ± 0.2
cantaloupe edible flesh		
control, no ClO_2	<LOD[c]	<LOD[d]
treated, ClO_2 (+) cantaloupe	<LOD[c]	<LOD[d]
cantaloupe edible flesh with rind		
control, no ClO_2	<LOD[c]	2.2 ± 0.2
treated, ClO_2 (+) cantaloupe	1319 ± 247	1.9 ± 0.3

[a]Data are means ± standard deviations of 3 replicates. [b]A nominal value is shown. Two of three replicates had tomato puree values less than the limit of detection (36 ng/g). A single replicate (shown) had chlorate residues above the LOD, but below the limit of quantitation (60 ng/g). [c]Limit of detection for chlorate in cantaloupe edible flesh was 15 ng/g; the limit of quantitation was 30 ng/g. [d]Limit of detection for perchlorate in cantaloupe edible flesh was 0.3 ng/g; the limit of quantitation was 1.5 ng/g.

ng/g for the perchlorate assay. Chlorate was either absent, or was present at levels below the LOQ, in control tomatoes and cantaloupe. A single replicate of control tomato puree contained chlorate residues above the LOD. Treatment with ClO_2 did not cause quantifiable chlorate residues to be formed in tomato puree or in edible flesh of cantaloupe; the edible flesh + rind fraction of cantaloupe, however, contained chlorate residues (1,319 ± 247 ng/g). The high concentration of chlorate residue on rind (as compared to tank rinse) confirms the notion that as chlorine dioxide was being generated, the cantaloupe surface effectively functioned as an efficient chlorine dioxide sink. Similar to the preponderance of total radioactive residue measured on cantaloupe surfaces[18] after exposure to gaseous $^{36}ClO_2$, the data provides a rationale for chlorine dioxide efficacy when chlorine dioxide gas concentrations remain low: because the gas is attracted to the vegetable surface where microbes colonize, achievement of high gas concentration is not an absolute necessity.

Perchlorate residues were present in control and treated tomato puree (Table 3), but chlorine dioxide treatment did not (P = 0.28) increase perchlorate residues relative to the control tomatoes. Recovery of perchlorate fortified at 30 ng/g of tomato puree was 111.5 ± 2.7%. Measurable perchlorate levels in control tomatoes used in this study is not surprising as a US-FDA survey of 62 domestic tomato sets collected from across the United States and 8 tomato sets from Mexico (commonly consumed in the U.S.) contained an average of 13.7 ppb of perchlorate.[27] Tomatoes fumigated with chlorine dioxide in this study clearly did not contain perchlorate burdens that were different than background perchlorate levels in control tomatoes.

Perchlorate content (Table 3) of rind plus edible flesh (1.9 ± 0.3 ng/g) of fumigated cantaloupe did not differ (P = 0.20) from the perchlorate content of untreated rind plus edible flesh homogenates (2.2 ± 0.2 ng/g). Recovery of perchlorate from fortified blank matrices averaged 101.5 ± 3.4%. Previous measurements of perchlorate residues in cantaloupe have been quite variable, depending upon the source of cantaloupes. For example, Krynitsky et al.[45] measured a median concentration of 9.6 ng/g (range <2 to 18.2 ng/g; n = 11) of perchlorate in edible flesh of cantaloupe originating in the United States

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(Arizona). However, the same study showed that when the whole cantaloupe (edible flesh, rind, and seeds) was measured, median perchlorate concentrations more than doubled relative to the flesh alone (median 23.9 ng/g; range <2 to 39.3 ng/g; n = 11). A later study from the same laboratory[26] showed that "edible portions" of cantaloupe (source unknown) with seeds contained greatly variable concentrations of residue, ranging from 2.8 to 115 ng/g perchlorate.

Collectively, results obtained from this and previous studies[18,19] are highly consistent with the known principles of chlorine dioxide chemistry and the interactions of chlorine dioxide and chlorite with reductants present in biological materials. That is, in the presence of biological reductant, chlorine dioxide may function as a five electron oxidant:[46,47]

$$ClO_2 + 1e^- \rightarrow ClO_2^- + 2e^- \rightarrow OCl^- + 2e^- \rightarrow Cl^-$$

During sanitation of tomatoes and melons, and presumably other vegetable materials, chlorine dioxide will react very rapidly with amino acids[46,48] (tryptophan < tyrosine ≪ cysteine; k from 3.2×10^4 to 1×10^7 M^{-1} S^{1-}), glutathione[46] (k 5.8×10^2 M^{-1} S^{1-}), NADH[49] (k 7.6×10^6 M^{-1} S^{1-}), nucleotides[50] (guanosine 5′-monophosphate, k 4.5×10^2 M^{-1} S^{1-}), iron[47] (k 3.9×10^3 M^{-1} S^{1-}), and a variety of phenols[51] (k from 1.4×10^3 to 1.58×10^8 M^{-1} S^{1-}) to form chlorite ion.[46,51,52] Rate constants of these magnitudes clearly explain why chlorine dioxide did not accumulate in tanks that contained tomatoes or melons.

Once formed, chlorite ion is also subject to reduction by plant-based biomaterials, albeit at somewhat slower—but still relatively fast—rates. For example, chlorite was not measured as a residue on tomatoes[18] or melons[18,19] because chlorite is subject to chemical reduction by aldoses,[53] lignin-based phenol and nonphenolic aldehydes[52] (k 0.6 M^{-1} S^{1-} to 39 M^{-1} S^{1-}), cysteine[46] (k 3.4 M^{-1} S^{1-}), polysaccharides,[54] phenols,[55] proteins[55,56] and metal cations such as iron[47,57,58] (k 4.0×10^3 M^{-1} S^{1-}), all components of plant-based organic matter. Even when chlorine dioxide is used for industrial-scale bleaching of pulp wood, chlorite ion is considered a chemical intermediate[52] chloroxyanion species. The product of chlorite reduction, hypochlorite (OCl^-) is very short-lived, being rapidly reduced to chloride ion[46] given the thermodynamic stability of chloride ion (−I oxidation state) relative to hypochlorite (+I oxidation state).

The absence of chlorite residues on produce sanitized with chlorine dioxide is of considerable importance given previous reports describing chlorite as being absorbed and excreted intact in mammals[59,60] and the toxicological potential ascribed to the chlorite ion.[61] With the known propensity for chlorite to serve as an oxidizing agent (see previous discussion), it is not surprising that chlorite was not a measurable residue in chlorine dioxide (Cl^{4+}) treated tomatoes and cantaloupe. However, it is surprising that Abdel-Rahman et al.[59,60] reported chlorite's absorption and excretion in rats, especially considering data from animals dosed with sodium chlorate (Cl^{5+}). That is, the intermediate specie, chlorite (Cl^{3+}), was never present in tissues or urine from cattle,[62,63] swine,[64] broilers,[65] or rats[56] dosed with sodium ^{36}Cl-chlorate, even though its 6 electron reduction product, chloride ion (Cl^-), was always present. The methods[66] used by, and conclusions[59,60] of, Abdel Rahmen et al., who reported that chlorite is a stable residue in rats dosed with chlorine dioxide, chlorite, and chlorate have been refuted.[56] Instability of chlorite in biomatrices has been further demonstrated; for example 17.3 μg/mL of chlorite had a half-life of only 4.5 min in bovine ruminal fluid[67] and was a detectable, but transitory, metabolite of ^{36}Cl-chlorate in pure cultures of *E. coli*.[68] In the latter study, chlorite was only measurable by directly injecting culture fluid, without pretreatment, onto an ion chromatograph equipped with a radiochemical detector. Finally, the instability of chlorite in seemingly inactive or marginally reducing matrices such as surface, ground, and even tap waters has led the US Environmental Protection Agency to recommend that precautions (the addition of preservatives, protection from light, and refrigeration) be taken at sampling to ensure accurate analytical results during water analysis.[69]

Given the apparent instability of chlorite ion, a reasonable question is why chlorite has been reported as a stable residue in chlorine dioxide treated rats[59,60] and produce.[11,14,16,17] In the case of chlorine dioxide treated rats, Hakk et al.,[56] has provided convincing evidence that the differential precipitation and solubility methods employed[66] were inadequate to speciate and quantify chlorite, chloride, and chlorate. The amperometeric method used to quantify residues on produce, cited by Han et al.,[11] Netramai,[16] and Saschower[17] (APHA method 4500-ClO2 C-Amperometric Method I), indirectly measures chlorite, and measures chlorate by difference.[70] The method is no longer recommended by the US EPA because of poor selectivity and sensitivity as described in some detail by Hoehn et al.[70] Ion chromatographic methods used by Tsai et al.[71] and Trinetta et al.[14] for measurements in produce are quantitative and may also be specific depending upon the matrix. Tsai et al.[71] did not measure detectable (LOD 0.1 mg/kg) residues of chlorite on potatoes, but Trinetta et al.[14] documented nondetectable chlorite in rinsewater of tomatoes (LOD stated to be 0.01 mg/L) to over 1200 mg/kg (1.2 parts per thousand) of chlorite residues on alfalfa sprouts. Because Trinetta et al.[14] state that chlorite quantitation was by ion chromatography with UV detection (λ_{max} not provided), and because Trinetta et al. state that alfalfa sprouts were visibly damaged subsequent to chlorine dioxide treatment, it is possible that UV-absorbing interferences could have been measured in alfalfa sprout rinses, especially since the untreated controls would not have such damage. Alternatively, chlorite might accumulate and have sufficient stability for measurement in watery, nonacidic plants like alfalfa sprouts or lettuce. Although we did not formally assay for chlorite in tank rinses of cantaloupe or in tomato rinsewater, we did look for the appearance of chlorite in ion chromatograms of rinse waters and found no evidence for its presence (see supplementary chromatogram, Figure S1).

Results from this study suggest that under the proper conditions, slow-release chlorine dioxide gas formulations could be used to sanitize tomatoes or cantaloupes with minimal deposition of perchlorate and chlorate residues on edible plant fractions. The data suggest that slow-release chlorine dioxide sanitation could be extended to other crop groups with minimal impact on food quality due to the presence of chloroxyanion residues.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.5b04153.

Representative ion chromatograms showing the apparent absence of chlorite in tomato rinse waters (PDF)

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Notes

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