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Distribution, Identification, and Quantification of Residues after Treatment of Ready-To-Eat Salami with ^{36}Cl-Labeled or Nonlabeled Chlorine Dioxide Gas

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ABSTRACT: When ready-to-eat salami was treated in a closed system with ^{36}Cl-labeled ClO_2 (5.5 mg/100 g of salami), essentially all radioactivity was deposited onto the salami. Administered $^{36}ClO_2$ was converted to ^{36}Cl-chloride ion (>97%), trace levels of chlorate (<2%), and detectable levels of chlorite. In residue studies conducted with nonlabeled ClO_2, sodium perchlorate residues (LOQ, 4 ng/g) were not formed when reactions were protected from light. Sodium chlorate residues were present in control (39.2 ± 4.8 ng/g) and chlorine dioxide treated (128 ± 31.2 ng/g) salami. If sanitation occurred under conditions of illumination, detectable levels (3.7 ± 1.5 ng/g) of perchlorate were formed along with greater quantities of sodium chlorate (183.6 ± 75.4 ng/g). Collectively, these data suggest that ClO_2 is chemically reduced by salami and that slow-release formulations might be appropriate for applications involving the sanitation of ready-to-eat meat products.

KEYWORDS: *chlorine dioxide, chlorite, chlorate, perchlorate, residue, sanitation*

INTRODUCTION

A growing body of evidence clearly demonstrates the efficacy of gaseous chlorine dioxide against pathogenic bacteria,[1] spoilage organisms,[2−4] spores,[5−7] molds,[8,9] and viruses[10,11] on a variety of surfaces[5,12,13] including produce[1] and spices.[14] Some organisms are remarkably sensitive to chlorine dioxide gas; for example, activity against airborne viral pathogens is maintained at concentrations below allowable time-weighted human exposure values[15−17] (i.e., 0.1 ppm). Given the variety of pests and pathogens and the ranges of concentrations at which chlorine dioxide gas is active, the gas has been proposed for a very large number of food safety and/or preservation applications.

Aqueous formulations of chlorine dioxide have been approved in numerous applications for sanitation of agricultural storage, handling, and transfer facilities.[13] In addition, aqueous chlorine dioxide is also approved for sanitizing poultry chiller and spray rinse waters.[13] Up to 3 mg/L chlorine dioxide in aqueous solution is approved by the U.S. Food and Drug Administration (FDA) for the treatment of raw poultry or as rinses of fruits and vegetables.[18] Limitations on the effectiveness of chlorine dioxide rinses exist, especially with respect to uses on food surfaces. For example, in poultry chiller rinses the maintenance of active levels of chlorine dioxide may be difficult, and poor permeation of liquids into cavities or porous surfaces may limit chlorine dioxide action on spoilage organisms and/or pathogens. In contrast, chlorine dioxide gas is easily dispersed in air and has an excellent ability to permeate spaces that may not be easily accessed by aqueous formulations. Nevertheless, to date, gaseous chlorine dioxide treatments have not been approved by regulatory organizations for applications involving microbiological decontamination of foods. One exception is the use of chlorine dioxide gas to prevent spoilage organisms on potatoes.[19]

A major limitation for the use of chlorine dioxide gas as a food preservative or a treatment to mitigate pathogen contamination has been a lack of definitive studies that demonstrate the fate and distribution of residues formed during chlorine dioxide gas sanitation. Previous studies have not necessarily been consistent with respect to the stability of putative chlorine dioxide reduction products. For example, metabolism and fate studies with labeled ^{36}Cl-chlorine dioxide gas in the late 1970s and early 1980s indicated that chlorine dioxide underwent a one-electron reduction in rodents to form chlorite ion (ClO_2^-) and that chlorite was sufficiently stabile to be excreted in urine.[20,21] In addition, studies with ^{36}Cl-labeled chlorite[22−24] or chlorate[21,22] in rats suggested that chlorite would be of toxicological concern if formed from either chlorine dioxide or chlorate. Later studies,[25] however, were unable to replicate the analytical methods used by Abdel-Rhaman[20−22] and Scatina.[23,24] Furthermore, using ion chromatography with radiochemical detection, Hakk et al.[25] were unable to measure chlorite residues in urine of rats treated with ^{36}Cl-chlorate. Similarly, Smith et al. were not able to detect chlorite residues in tissues or excreta of cattle,[26,27] swine,[28] or broilers[29] dosed with ^{36}Cl-chlorate. Additionally, Smith et al.[30] were unable to recover any ^{36}Cl-chlorite residue after direct administration of >100 μg/g sodium ^{36}Cl-chlorite into tomatoes, indicating that chlorite is unstable, at least in tomato matrix. In contrast, Smith et al.[31] were able to detect the *transient* formation of ^{36}Cl-chlorite from ^{36}Cl-chlorate in pure cultures of *Escherichia coli*. Furthermore, chlorite residues were not measurable after treatment of tomatoes[30] or cantaloupe[30,32]

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with ^{36}Cl-labeled chlorine dioxide gas. The fact that chlorite is difficult to measure after chlorine dioxide treatment is consistent with its reactivity toward the plethora of reductants present in biological materials.[33]

Transformation products that have been reproducibly measured after exposure of fresh produce to ^{36}Cl-chlorine dioxide gas are ^{36}Cl-chloride, ^{36}Cl-chlorate, and ^{36}Cl-perchlorate. By far, the major transformation product of chlorine dioxide is chloride ion, with lesser amounts of chlorate ion formed.[30,32] Chlorate may be formed as a disproportionation product,[34,35] whereas perchlorate formation is a byproduct of gas-phase reactions catalyzed by light.[30] Indeed, the formation of both chlorate and perchlorate can be prevented by protecting gas-phase chlorine dioxide from light.[30,33] In practice, unwanted residues related to chlorine dioxide sanitation may be minimized or eliminated completely simply by conducting fumigations in the dark.

One application of chlorine dioxide gas that has not been investigated in great depth is its potential activity against pathogens, namely *Listeria monocytogenes*, on precooked ready-to-eat meat products. Although many ready-to-eat meat products are precooked or processed in a manner that will kill pathogens (i.e., pressure, fermentation, drying), recontamination during peeling, slicing, and/or repackaging may occur at the retail level.[36] Contamination with *Listeria* is a major issue for ready-to-eat meat products because of its ubiquitous presence in the environment; the potential for contamination after initial processing; its ability to propagate, even at cold environmental temperatures;[36] and the severity of pathologies associated with human infection.[37] Because chlorine dioxide is active against *L. monocytogenes* on a number of surfaces,[38−40] we anticipate that it would be active on the surfaces of ready-to-eat meat surfaces. Therefore, the purpose of this study was to determine the disposition and chemical fate of ^{36}Cl-labeled chlorine dioxide gas on treated salami and to determine oxidative residues of chlorine dioxide gas in ready-to-eat salami after treatment with unlabeled gas.

MATERIALS AND METHODS

Radiochemical Study. *Radiochemicals.* Radioactive chlorine dioxide ($^{36}ClO_2$) was generated by the mineral acid catalyzed oxidation of $Na^{36}ClO_2$ as generally described by Smith et al.[30] Two radiolabeled experiments were conducted. For experiment A, 378 μL of stock $Na^{36}ClO_2$ (345 μg; 13,920 dpm/μg) was combined with 36 μL of sodium chlorite technical solution (318 mg/mL), to a sodium chlorite specific activity of 408 dpm/μg, and 87 μL of purified water in a Tyvek sachet (3 × 17 cm). Acidification of the chlorite solution with 1.8 M HCl (250 μL) initiated the release of $^{36}ClO_2$. The specific activity of the $^{36}ClO_2$ gas was 547 dpm/μg. For experiment B, 194 μL of stock $Na^{36}ClO_2$ (257 μg; 13,920 dpm/μg) was combined with 36 μL of sodium chlorite technical solution (318 mg/mL), to a sodium chlorite specific activity of 306 dpm/μg, and 170 μL of purified water in a Tyvek sachet (3 × 17 cm). Acidification of the chlorite solution with 1.8 M HCl (250 μL) initiated the release of $^{36}ClO_2$. The specific activity of the resulting $^{36}ClO_2$ gas was 410 dpm/μg.

Salami Treatment. Salami (Genoa 019; water, 42.3%; total fat, 30.97%; protein, 18.39%; carbohydrate, 3.27%; sugar, 3.19%; sodium, 1.6%; fiber, 0.03%; calcium, 0.03%) was obtained from the Patrick Cudahy Co. (Cudahy, WI, USA). Two $^{36}ClO_2$ fumigation experiments (A and B) were conducted, each with approximately 100 g of fresh-cut (~12 cm diameter × 1.5 cm thickness) salami with target quantities of 5.5 mg of $^{36}ClO_2$ gas generated during 2 h treatment periods. Fumigations occurred within 5.7 L (11 × 22 × 23.5 cm; W × L × D) sealable glass tanks essentially as described by Smith et al.[30] except that fumigation tanks and lids were each protected from laboratory illumination by an aluminum foil wrap.

After the reaction chamber had been sealed, duplicate 5 mL aliquots of chamber gas were removed through butyl septa placed within the glass lids at 5, 10, 15, 30, 45, 60, 90, and 120 min with a gastight syringe. Collected gas was immediately bubbled through 3 mL of 0.1 M sodium thiosulfate within glass liquid scintillation counting (LSC) vials. Liquid scintillation fluid (15 mL; Ultima Gold; PerkinElmer Life Science) was added to each vial, and radiochlorine was determined by LSC (PerkinElmer, TriCarb 2910; 20 min counts). Background was quantified using quintuplicate 3 mL aliquots of untreated 0.1 M sodium thiosulfate.

Recovery of Chamber Gas Radioactivity. At the end of each experiment unreacted ^{36}Cl-chlorine dioxide was trapped into 2 L of 0.1 N sodium thiosulfate by pumping room air (1.0 h; approximately 11 chamber volumes) through the exposure chambers. Air was pumped through coarse micro gas dispersion tubes (Chemglass Life Sciences, Vineland, NJ, USA) submerged in the thiosulfate solution. The trapping solution was mixed by inversion 10 times, quintuplicate 3 mL aliquots were placed in glass LSC vials, and LSC fluid was added. Triplicate 3 mL aliquots of blank 0.1 M thiosulfate were also pipetted into respective 20 mL glass LSC vials to quantify background activity. Thiosulfate samples were counted for 20 min each to determine residual gaseous chlorine dioxide.

Quantification Radiochlorine on Salami. Salami was removed from the sanitation chamber and placed in a Waring blender. A known mass of purified water (100 g) was added to the salami, and the salami was homogenized until a puree was obtained. Quintuplicate 0.5 g aliquots of the blended ready-to-eat salami were placed in glass LSC vials, and 2 mL of 1 M NaOH was added. Each sample was digested overnight at 70 °C using a water bath. After digestion, 15 mL of Ultima Gold LSC fluid was added to each vial. Total radiochlorine in the salami was determined by LSC for a minimum of 20 min each. Quintuplicate vials containing 0.5 g of untreated, pureed control salami were used to determine background radioactivity. Aliquots (50 mL) of the remaining homogenate were placed into polypropylene tubes (50 mL) and frozen (−20 °C) until further analysis.

Recovery of Chamber Residual Radioactivity. Stir bars and glass pedestals[30] were removed from the sanitation chambers, placed in glass beakers, and rinsed sequentially with purified water a minimum of three times. Sequential rinses were placed into a 1 L volumetric flask. Reaction chamber lids were rinsed into the reaction tank, and reaction chambers were rinsed using a squeeze bottle containing approximately 500 mL of purified water. Sequential rinses of approximately 100 mL were combined into the 1000 mL volumetric flask containing rinsewater from the stir bars and glass pedestals. After filling to the mark with purified water, the volumetric flask was mixed by inversion (10 times), triplicate archival aliquots were removed, and quintuplicate 2 mL aliquots were analyzed by LSC (20 min).

Radioactivity remaining within gas generation sachets was recovered after sequential rinsing with purified water and transfer of the rinse fractions into a 0.5 L volumetric as described for the tank rinse. Sachet radioactivity not removed by the water rinse was quantified after cutting each sachet into approximate 1 cm strips and counting each strip directly by LSC.

Preparation of Salami Solid and Liquid Fractions. Control and test salami homogenates were thawed at room temperature, and duplicate 5 g aliquots were weighed into respective 50 mL polypropylene centrifuge tubes. Aliquots (5 mL) of hexane were added to each tube, and tubes were mixed for 15 min using a Rotamix (ATR, Laurel, MD, USA) mixer. Tubes were centrifuged for 20 min at 30000*g* using a Sorvall RC5B Plus centrifuge. The hexane phase was removed from each tube and placed into respective 10 mL volumetric flasks. Hexane extractions were repeated with an additional 4 mL of hexane, extracts were combined, and volumetric flaskswere diluted to the mark with hexane. After thorough mixing by inversion (10 times), triplicate 2 mL aliquots of the hexane fraction were removed and placed in respective 20 mL glass LSC vials for radiochlorine quantitation (20 min) using hexane blanks to determine background (n = 5).

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After extraction with, and removal of, hexane, the aqueous layer of each tube was transferred to respective 10 mL volumetric flasks and diluted to the mark with purified water. Aqueous fractions were mixed thoroughly by inversion (10 times), and triplicate 2 mL aliquots were placed in respective 20 mL glass vials for LSC (20 min) using water blanks (n = 5) to determine background.

The remaining pellet fraction was mixed with a glass rod, and triplicate 0.25 g aliquots of each pellet were placed into respective 20 mL glass vials. Pellet aliquots were digested overnight (70 °C) in 2 mL of 1 M NaOH using a preheated water bath. After digestion, 15 mL of Ultima Gold LSC fluid was added to each vial while still warm; vials were allowed to dark-adapt for at least 1 h prior to the commencement of LSC (20 min).

Aqueous Extraction of Pellet Radioactivity. Aliquots (0.5 g) of the pellet fraction were removed and placed in 50 mL round-bottom centrifuge tubes. Purified water (5 mL) was added to each tube, and pellets were suspended by ultrasonic agitation for 30 s. Thereafter, tubes were mixed for 15 min using a Rotamix mixer and subsequently centrifuged for 20 min at 30000*g*. The resulting aqueous phases were transferred into respective 20 mL glass LSC vials, and extract weights recorded. Extractions were repeated on pellets two more times for a total of three aqueous extractions per pellet. Aliquots (1 mL) of each extraction were transferred to vials, diluted with LSC fluid (15 mL), and, after dark adaptation (1 h), samples were counted for 20 min each.

Radiochemical Analysis of Salami Extracts. Identification of radioactive residues was accomplished by fortification of filtered (0.45 μm PTFE) salami aqueous aliquots (0.2 mL) with nonradioactive chlorite, chloride, and chlorate (42−43 μg; for use as chromatographic markers) and separation of ions using ion chromatography. Chlorite, chloride, and chlorate fractions were then collected into separate LSC vials as they eluted from the detector, and ^{36}Cl eluting in each fraction was subsequently determined by LSC (20 min). Recoveries of radioactivity from the chromatograph were determined by also quantifying the total amount of radiochlorine in triplicate 0.2 mL aliquots of each aqueous salami extract. Chromatography was performed on a Waters 600 chromatograph fitted with PEEK pump heads and tubing. The mobile phase was 10 mM NaOH pumped isocratically at 1 mL/min through a Dionex AS11-HC (4 × 250 mm) column. A conductivity detector (Dionex CD-25; 0.1 V, 100 mA, range 3000) with external water suppression (Dionex ASRS 300; 4 mm) was used to monitor the elution of sample components. Guard columns were not used for the chlorate analysis. ^{36}Cl-perchlorate residues in salami aqueous extracts were determined as described for chlorate except that AG- and AS-16 HC columns were used with a mobile phase of 50 mM NaOH and a flow rate of 1.0 mL/min.

Determination of Choroxyanions in Tank Rinse Water. Chlorate and perchlorate in rinsewater were determined using a Dionex ICS2100 chromatography system using the conditions previously described.[33] The limits of detection for chlorate and perchlorate were 0.5 and 2 ng/mL, respectively; the limits of quantitation for chlorate and perchlorate were 5 and 4 ng/mL, respectively.

Half-Life Calculation. Chlorine dioxide half-lives in exposure tanks were fit to a one-phase exponential decay curve ($Y = Y_0^{-KX}$) using least-squares nonlinear regression, where Y is $^{36}ClO_2$ concentration, K is the least-squares rate constant, and X is time in minutes. Calculations were performed using GraphPad 6.05 for Windows (GraphPad Software, La Jolla, CA, USA).

Nonlabeled Residue Study. *Chemicals.* Chlorine dioxide was generated using ICA Trinova (Newnan, GA, USA) ZC-series dry media, which, unlike the mineral acid catalyzed release of chlorine dioxide used in the total residue study, is designed to slowly release ClO_2 at defined rates in a user-friendly and safe formulation. Sodium [$^{18}O_4$]perchlorate internal standard was purchased from Icon Isotopes (Summit, NJ, USA), and sodium [$^{18}O_3$]chlorate internal standard was prepared in-house.[41] Purified water (>18 mΩ cm) was generated using a Milli-Q Water System (Millipore, Darmstadt, Germany).

Salami Treatment. Experiments were conducted under four sets of conditions described in Table 1. Negative controls contained salami and no ClO_2-generating system. Chlorine dioxide controls measured chlorine dioxide release from the ZC-series dry media in tanks that were protected from light, but which contained no salami. Two sets of tanks were used to measure the formation of chlorate and perchlorate on salami after sanitization with ClO_2, one set under darkened conditions and the other under laboratory illumination. Within day, each treatment was replicated once; the experiment was replicated three times on separate days.

Table 1. Test Conditions for the Nonlabeled Salami Residue Study

		variable		
test conditions	n	salami	ClO_2	light
negative control	3	yes	no	no
chlorine dioxide control	3	no	yes	no
chlorine dioxide sanitation−dark	3	yes	yes	no
chlorine dioxide sanitation−light	3	yes	yes	yes

Experiments were conducted within 37.5 L (24.5 × 50.2 × 30.5 cm; W × L × D) glass aquaria sealed with Plexiglas lids as described.[30] With the exception of the tanks designated for exposure to light, tanks and lids were each wrapped with aluminum foil to protect sanitation chambers from illumination. Each tank was supported by two magnetic stir plates and was equipped with a temperature/humidity probe (PN 14-649-84; Fisher Scientific, Waltham, MA, USA), two magnetic stir bars, and a Plexiglas lid equipped with a 1.3 cm gas sampling port stoppered with a silicone grease-lined butyl stopper. Within each tank, two aliquots (102 ± 6 g) of fresh salami (Genoa 019, Patrick Cudahy) were suspended from each of three Plexiglas strips braced onto the inner strip of each tank (n = 6 salami aliquots per tank). Salami slices were suspended in the tank using fishing hooks and line (Eagle Claw, Denver, CO, USA). Total weights of salami for each tank averaged 614 ± 22 g and did not differ (P = 0.61) between treatments. After the placement of salami into each tank, magnetic stir bars were activated to promote the circulation of gases. Preweighed aliquots of ZC-series media A and B were then mixed within Tyvek sachets to release 55 mg/kg of salami over a 6 h period. Upon mixing, sachets were immediately placed into sanitation chambers and the chambers sealed. For the negative control treatment (no gas), appropriate aliquots of ZC-series media A and B were weighed, added to separate Tyvek sachets, and placed separately (not mixed) into the reaction chambers.

Temperature and humidity were recorded for each chamber at 0, 15, 30, 45, 60, 90, 120, 240, and 360 min after the start of an experiment. Aliquots (10 mL) of headspace gas were removed from each tank using a 10 mL gastight syringe at 0, 15, 30, 45, 60, 90, 120, 240, and 360 min to determine chlorine dioxide concentration. Illumination was measured at 0 and 360 min using an Extech light meter (PN 401025; Extech, Nashua, NH, USA).

At the end of the 6 h test period, tanks were opened and two of the six salami slices were removed for independent chemical analysis. Selected salami pieces separately weighed, cubed, and placed into a homogenizer (Oster model BCCG08 with a no. 4888 jar; Boca Raton, FL, USA). Equal weights of water were added to each salami slice, and salami was homogenized to a smooth puree. Aliquots (~50 g) of the salami puree were placed in 50 mL polypropylene tubes (Sarstedt 62.5447.004, Nümbrecht, Germany) and stored at −20 °C or less until analyses.

Each reaction chamber was rinsed sequentially with aliquots of purified water. For each tank, sequential rinse aliquots were pooled into a 500 mL volumetric flask, the pooled aliquots were diluted to the mark with purified water, and the contents of the flask were mixed by inversion a minimum of 10 times. Aliquots (12 mL) were removed, placed into 15 mL polypropylene tubes (Sarstedt 62.554.002), and stored at −20 °C or less until analysis.

Sample Extraction. Salami homogenates were thawed, and aliquots (10 g) were placed into 50 mL, round-bottom centrifuge tubes (Thermo Scientific, Waltham, MA, USA). Sample sets included controls (blanks), fortified controls, and chlorine dioxide-treated salami. Fortification samples were spiked with 10 μL of a 10,000 ng/mL sodium chlorate solution, equivalent to 20 ng of sodium chlorate

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per gram of unprocessed salami (10 ng/g homogenate). All tubes were fortified with 39 μL of 6500 ng/mL $NaCl^{18}O_3$, equivalent to 50 ng/g of unprocessed salami (25 ng/g homogenate). After mixing, 10 mL of hexane was added to each tube, the tubes were mixed by rotation (Rotamix; Appropriate Technical Resources, Laurel, MD, USA) for 15 min. and were centrifuged (Sorvall RCB5; Thermo Fisher Scientific, Waltham, MA, USA) for 20 min at 30000*g*. The hexane layer was removed using a Pasteur pipet and discarded. After resuspension of the pellet, the hexane extraction was repeated. The second hexane extract was removed, and the aqueous phase was placed into a weighed 15 mL polypropylene tube and the total weight recorded.

Dual solid phase extraction (SPE) tubes were conditioned using acetonitrile and water. Carbon-based SPE tubes (ENVI-Carb; 0.55 g, 6 mL; Supelco, Bellefonte, PA, USA) were conditioned with 6 mL each of acetonitrile and water, whereas silica-based SPE tubes (Bond-Elut Certify; 1 g, 6 mL; Agilent, Lake Forrest, CA, USA) were conditioned with 2 mL each of acetonitrile followed by purified water. Aqueous extracts (80% of the total collected) were loaded onto the ENVI-Carb SPE tubes, and the eluent was allowed to flow onto the Bond-Elut SPE tubes via gravity flow. Eluent leaving the Bond-Elut tubes was collected into clean 5 mL volumetric flasks, and the tubes were rinsed with 2 mL of purified water. Because gravity flow was employed, the volume of the volumetric flasks was not exceeded. Volumetric flasks were diluted to the mark with purified water, capped, and mixed by inversion 10 times. Aliquots (~3 mL) were sequentially filtered through 0.45 and 0.2 μM filters into 1 mL sample and archive vials, respectively.

Sodium perchlorate residues in salami were measured as described for sodium chlorate except that blank sample aliquots were fortified with 100 ng of sodium perchlorate (20 ng/g unprocessed salami) and all samples were fortified with 250 ng of $NaCl^{18}O_4$ internal standard (50 ng/g unprocessed salami).

Analysis of Chlorine Dioxide. Preparation of chlorine dioxide standard and measurement of chlorine dioxide release during the sanitation process was conducted as previously described.[33]

Chlorate and Perchlorate in Rinse Water. Chlorate and perchlorate ions in rinse waters were quantified as previously described for rinse waters.[33] For the analysis of tank rinsewater from this study, the method limits of detection (LOD) and quantification (LOQ) for sodium chlorate were 2.4 and 5.0 ng/mL, respectively. The same measures for sodium perchlorate were 10.0 and 4.1 ng/mL, respectively.

Chlorate Analysis in Salami Extracts by LC-MS/MS. A matrix-matched calibration curve consisted of points at 0, 5, 10, 20, 40, 60, 80, and 100 ng/mL of sodium chlorate. An Acquity UPLC system equipped with a Waters triple-quadrupole mass selective detector was used to quantify chlorate in salami extracts. Selected ion chromatograms were constructed for the m/z 88.8 → 70.8 transition of the ^{35}Cl-isotope of the $Cl[^{18}O_3]$-labeled chlorate internal standard and for the m/z 82.8 → 66.8 transition of the ^{35}Cl-isotope of native chlorate for both the matrix-matched standards and unknowns. The ^{37}Cl-isotope transitions at m/z 90.8 → 72.8 and m/z 84.8 → 68.8, for the internal standard and native chlorate, respectively, were used for confirmatory purposes. Sodium chlorate residues were quantified in unknowns based on regression of the ion ratio of the internal standard and native chlorate with concentration of sodium chlorate in standards. Sample aliquots (20 μL) were injected from an autosampler maintained at 5 °C onto a Dionex (Thermo Scientific) IonPac AS21 column (2 × 250 mm) protected by an AG21 guard column (2 × 50 mm) at ambient temperature. Sodium chlorate was eluted with an isocratic mobile phase of 180 mM methylamine in water at a flow rate of 0.35 mL/min. Ions were detected in the negative ion mode with a capillary voltage of 0.6 kV and a cone voltage of 45 V for ClO_3^- and 50 V for the $Cl[^{18}O_3^-]$ internal standard; the source and desolvation temperatures were set at 120 and 400 °C, respectively, with cone and desolvation gas flows at 60 and 800 L/h, respectively. Argon was used as the collision gas at a flow rate of 0.15 mL/min with collision energy set at 15 eV.

Because chlorate concentrations for some salami samples exceeded the highest point of the calibration curve during the initial analysis, sample dilutions were required. For those samples, dilutions (1/10 to 1/15) were made by pipetting 1 part of sample extract into the 9 parts of purified water or 1 part of sample extract into 14 parts of purified water. To control for matrix effects, matrix-matched standards were also diluted in a similar manner.

The 5 ng/mL matrix-matched standard routinely provided a signal-to-noise ratio >5, so the instrument LOQ corresponded to the 5 ng/mL matrix-matched standard. A corresponding method LOQ of 6 ng/g resulted when the original sample mass and dilution were accounted for; the method LOD was arbitrarily set at half the LOQ to 3 ng/g.

Perchlorate Analysis in Salami Extracts by LC-MS/MS. Perchlorate concentrations in aqueous salami extracts were determined as described for chlorate except that samples were fortified with 25 ng of $Cl[^{18}O_4]$-labeled perchlorate internal standard. Samples were quantified using a matrix-matched standard curve consisting of points at 0, 10, 20, 40, 60, 80, and 100 ng/mL of sodium perchlorate containing 50 ng/mL of internal standard. Selected ion chromatograms were constructed for the m/z 106.5 → 88.7 transition of the ^{35}Cl-isotope of the $Cl[^{18}O_4]$-labeled perchlorate internal standard and for the m/z 98.7 → 82.7 transition of the ^{35}Cl-isotope of native perchlorate for both the matrix-matched standards and unknowns. The ^{37}Cl-isotope transitions at m/z 108.5 → 90.5 and m/z 100.7 → 84.7, for the internal standard and native perchlorate, respectively, were used for confirmatory purposes. Chromatographic conditions were the same for the chlorate analysis. Ions were detected in the negative ion mode with a capillary voltage of 2.6 kV and a cone voltage of 65 V for both ClO_4^- and the $Cl[^{18}O_4^-]$ internal standard; the source and desolvation temperatures were set at 150 and 500 °C, respectively, with cone and desolvation gas flows at 50 and 800 L/h, respectively. Argon was used as the collision gas at a flow rate of 0.15 mL/min with collision energy set at 15 or 20 eV.

RESULTS AND DISCUSSION

Radiochemical Studies. *Release of Radioactive Chlorine Dioxide Gas.* Figure 1 clearly shows that ^{36}Cl-chlorine dioxide gas was generated by the mineral acid catalyzed oxidation of sodium ^{36}Cl-chlorite and that gas was produced fairly rapidly with maximal gas concentrations measured at the 15 min sampling time for both experiments. Thereafter, gas concentrations decreased throughout the 2 h exposure period to levels just above the reliable radiochlorine quantification limits (i.e., 4 dpm above background). The estimated chlorine dioxide half-lives for experiments A and B were 27.7 and 17.7 min, respectively, as calculated from the peak concentration at 15 min. Maximum gas concentrations represented only 24−39% of the 5.5−5.7 mg target chlorine dioxide mass. Because the salami was serving as a ClO_2 sink, maximal gas concentrations are a poor indicator of the total $^{36}ClO_2$ generated. A blank tank was

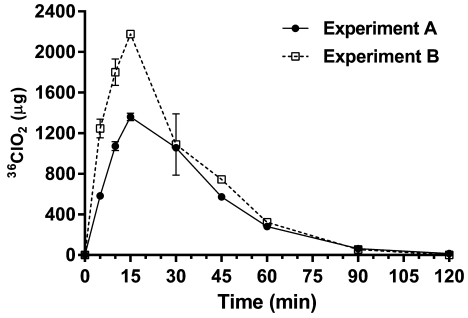

Figure 1. ^{36}Cl-chlorine dioxide gas concentration profiles during the treatment of ready-to-eat salami. Reaction tank volumes were 5.7 L; gas samples were removed at the indicated time points and bubbled through thiosulfate, and radiochlorine in the thiosulfate was quantified by liquid scintillation counting.

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not run because of a limited quantity of ^{36}Cl-chlorite. Nevertheless, the gas measurements clearly indicate that once $^{36}ClO_2$ was generated, the gas did not accumulate and that the salami efficiently removed gaseous ClO_2 from the treatment chambers.

The overall distributions of radioactivity in the test systems of experiments A and B are shown in Table 2. After a 2 h sanitation period, trivial quantities of $^{36}ClO_2$ gas remained in reaction chambers as evidenced by the lack of radioactivity within the thiosulfate traps. Condensation of gaseous residues on the reaction chamber walls was minimal (0.3−0.4%) relative to condensation that occurred in test systems containing tomatoes or cantaloupe (1.7−12.5%) exposed to light.[30] Radioactivity was nearly quantitatively recovered; the salami homogenate and the reaction sachets collectively contained 99.2 and 95.6% of the system radioactivity for experiments A and B, respectively. If one discounts the radioactivity associated with the reaction sachet rinse and the sachet itself (radiochlorine not converted to $^{36}ClO_2$), the entire amount of ^{36}Cl-chlorine dioxide gas generated in both experiments A and B was consumed by the salami. That is, for experiments A and B, respectively, radioactivity deposited onto salami represented 5.7 and 5.6 mg of $^{36}ClO_2$ gas equivalents, which was within 5% of the target treatment of 5.5 mg $^{36}ClO_2$.

Table 2. Distribution of Radioactivity in the ^{36}Cl-Chlorine Dioxide Sanitation Test Systems[a]

system component	expt A	expt B
sodium thiosulfate trap	0.0	0.0
reaction chamber rinse	0.4	0.3
reaction sachet rinse	41.2	38.7
reaction sachet	0.4	0.6
salami homogenate	58.0	56.9
total recovery	100.0	96.5

[a]Data are expressed as the percentage of starting radioactivity.

Table 3 shows the distribution of radioactive residues deposited onto salami. A small (≤1%) but measurable quantity of the salami radiochlorine was hexane extractable. Back extraction of the hexane fraction with water did not remove the radioactivity (94.4 ± 3.8% of the hexane radioactivity remained in the hexane fraction). Incorporation of radiochlorine into unsaturated fats is likely the source of the hexane-extractable radioactivity. For example, Ghanbari et al.[42] measured the incorporation of chlorine dioxide into a variety of unsaturated fats including oleic, linoleic, linolenic, and arachidonic acids. The great majority (~94%) of the salami radioactivity was associated with the aqueous fractions (supernatant and rinses), with most radioactivity being present in the salami supernatant and the first aqueous pellet extract. Such a distribution would be consistent with the formation of inorganic ions from chlorine dioxide. About 5% of the total salami radioactivity was not recovered in either the hexane or aqueous extracts and remained with the extracted pellet. Experimental recoveries of the radioactivity deposited onto salami were excellent at 100.3 and 99.9% for experiments A and B, respectively.

Radiochemical compositions of aqueous salami extracts are shown in Table 4. That is, chlorine dioxide oxidation (chlorate, perchlorate) or reduction (chloride, chlorite) products of potential toxicological concern were determined in the aqueous extracts. In experiment A, the only radioactive residue detected in the aqueous supernatant and the first water extract was ^{36}Cl-chloride ion, the penultimate reduction product of chlorine dioxide. In experiment B, both chlorite (ClO_2^-) and chlorate (ClO_3^-) were present at trace levels in both the initial aqueous supernatant and the first water extract. As shown in Table 3, the relative variation in both chlorite and chlorate among replicates (n = 6 analyses for each fraction of experimentd A and B) was great (coefficients of variation = 23−200% for chlorite and chlorate, respectively) because both analytes were present at levels just above background. ^{36}Cl-perchlorate residues were not detectable in salami extracts of either experiment.

Table 3. Extraction and Distribution of Radioactive Residues within Salami Sanitized with $^{36}ClO_2$ after Homogenization and Extraction[a]

fraction	expt A	expt B
hexane	0.7	1.0
aqueous		
supernatant	42.0	43.5
pellet extract 1	45.8	44.3
pellet extract 2	4.8	4.6
pellet extract 3	1.6	1.3
total aqueous[b]	94.2	93.7
extracted pellet	5.4	5.2
total recovery[c]	100.3	99.9

[a]Data are expressed as percentages of the total salami radioactivity present in each fraction. [b]Aqueous radioactivity is calculated as the summation of radioactivity present in the aqueous supernatant and in the aqueous supernatants resulting from pellet extractions 1, 2, and 3. [c]Total radioactivity is calculated as the summation of radioactivity present in the hexane, total aqueous, and extracted pellet fractions.

Table 4. Radiochemical Composition of Residues Present in Supernatant and Aqueous Extracts of Salami Sanitized with $^{36}ClO_2$[a]

fraction	anion	expt A	expt B
supernatant	ClO_2^-	0.0 ± 0.0	1.2 ± 1.3
	Cl^-	108.3 ± 12.5	101.6 ± 4.0
	ClO_3^-	0.0 ± 0.0	2.2 ± 0.5
	ClO_4^-	0.0 ± 0.0	0.0 ± 0.0
recovery		108.3 ± 12.5	104.9 ± 5.2
pellet extract 1	ClO_2^-	0.0 ± 0.0	1.5 ± 2.9
	Cl^-	99.8 ± 5.2	97.2 ± 4.3
	ClO_3^-	0.3 ± 0.7	2.3 ± 4.6
	ClO_4^-	0.0 ± 0.0	0.0 ± 0.0
recovery		100.1 ± 5.5	101.0 ± 5.6
pellet extract 2	insufficient activity for characterization		
pellet extract 3	insufficient activity for characterization		

[a]Data are presented as mean percentage ± standard deviation of recoveries of total injected radioactivity in each anion fraction (n = 6 per chromatographic analyses per experiment per extract).

Residue Study. Table 5 shows the temperature and humidity changes that occurred within sanitation tanks during the 6 h experiments. Temperatures across all treatments were similar (P > 0.05) during the initial 45 min of study, but increased (P < 0.05) in illuminated tanks during the treatment

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Table 5. Temperature and Relative Humidity in Test and Control Tanks during Chlorine Dioxide Sanitation of Ready-To-Eat Salami[a]

	temperature (°C)			relative humidity (%)		
	ClO_2		no ClO_2	ClO_2		no ClO_2
time (min)	light	dark	dark	light	dark	dark
0	23.4 ± 1.8	22.2 ± 1.9	23.1 ± 1.5	32 ± 10x	45 ± 12y	35 ± 12x
15	23.2 ± 2.3	22.1 ± 1.8	22.1 ± 1.4	57 ± 2	66 ± 1	62 ± 8
30	24.3 ± 2.2	22.3 ± 1.6	22.2 ± 1.5	66 ± 3	73 ± 2	66 ± 14
45	25.3 ± 2.0	22.6 ± 1.5	22.4 ± 1.6	72 ± 4	78 ± 2	75 ± 6
60	26.0 ± 1.8x	22.8 ± 1.6y	22.6 ± 1.6y	77 ± 5	83 ± 1	79 ± 3
90	26.7 ± 1.6x	23.2 ± 1.6y	22.9 ± 1.6y	87 ± 4	89 ± 1	86 ± 2
120	27.0 ± 1.4x	23.4 ± 1.5y	23.1 ± 1.6y	92 ± 3	93 ± 1	90 ± 2
240	27.4 ± 1.3x	23.7 ± 1.4y	23.3 ± 1.5y	94 ± 3	99 ± 1	92 ± 8
360	27.5 ± 1.2x	24.0 ± 1.5y	23.6 ± 1.7y	94 ± 3	99 ± 0	90 ± 12

[a]Means ± standard deviations of three observations per treatment. Means within row and variable (temperature or humidity) having differing letters (x, y) differ ($P < 0.05$).

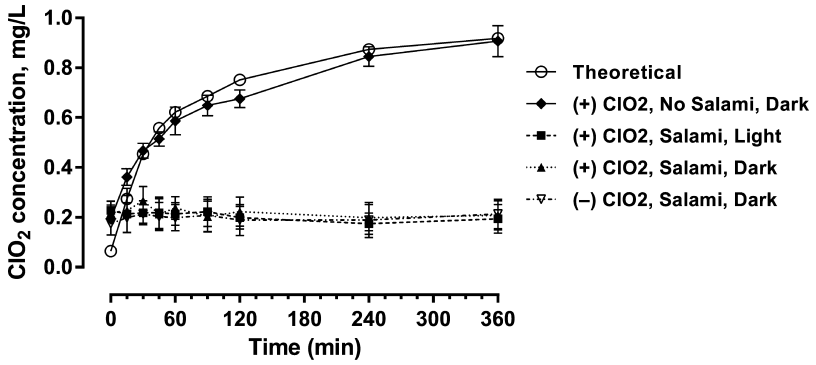

Figure 2. Theoretical (open circles) chlorine dioxide accumulation; chlorine dioxide accumulation in the absence of salami and light (solid diamonds), in the presence of salami and light (solid squares), in the presence of salami and in the dark (solid triangles), and in the absence of a chlorine dioxide generating system (open inverted triangles).

period. Temperatures within the tanks having no illumination did not differ ($P > 0.05$) during the chlorine dioxide fumigation period. Across all tanks, there was a significant increase ($P < 0.05$) in relative humidity during the 6 h treatment period. However, only at the beginning of the fumigation (0 min) was there a difference ($P < 0.05$) in humidity among treatments; salami exposed to ClO_2 in darkness had a higher humidity than that from the other tanks. By the 15 min measurement, humidity differences did not occur ($P > 0.05$). Light intensities on the outside of darkened reaction chambers averaged 5 ± 1 lx for experiments run in the dark and 1110 ± 46 lx for illuminated experiments.

Figure 2 shows chlorine dioxide accumulation in control (no salami) and experimental sanitation tanks. In the absence of salami, chlorine dioxide accumulation in the test system was nearly identical to the theoretical chlorine dioxide release profile of the media, derived from the manufacturer's technical specifications, adjusted for the tank volume used. However, when salami was present, chlorine dioxide accumulation did not occur, regardless of whether the treatments occurred in the dark or were illuminated. These data are entirely consistent with experiments A and B with $^{36}ClO_2$; radiolabeled experiments established that 98.5% of the radioactivity generated as chlorine dioxide (55 mg/kg dose) was present on the salami after a 2 h treatment. Both studies clearly indicate that salami has a very large capacity for chlorine dioxide adsorption.

Table 6 summarizes perchlorate and chlorate residues present in treated and untreated salami. Sodium perchlorate residues were not detectable (LOQ = 4 ng/g) in control (no ClO_2) salami or in test salami sanitized with ClO_2 in the dark. For salami sanitized with ClO_2 under conditions of laboratory illumination, measurable sodium perchlorate residues were present in two of three replicates, albeit at concentrations just above the method LOQ; as a consequence, the average concentration of perchlorate in the three illuminated samples was below the LOQ at 3.7 ± 1.5 ng/g (the sample containing nonquantifiable residues was included in the average at half the method LOQ).

In contrast, sodium chlorate residues were present (Table 6) in all samples tested, including salami that was not exposed to chlorine dioxide. Control salami averaged 39.2 ± 4.8 ng/g sodium chlorate, with sodium chlorate residues in light and dark ClO_2-treated salamis containing 183.6 ± 75.4 and 128.0 ± 31.2 ng/g, respectively. If one takes the sodium chlorate present in the control salami into account, then the light- and dark-reacted salamis averaged 144.4 and 88.8 ng/g, respectively.

In general, the residue pattern observed with the salami is consistent with residue patterns observed in vegetables and melons after gaseous chlorine dioxide treatment.[33] That is, chlorate residues had a greater propensity to form from chlorine dioxide than perchlorate residues, and exposure to light tended to enhance the formation of both chlorate and perchlorate residues.[30] In this study, perchlorate residues were present only (above 4 ng/g) in salami treatments exposed to light (two of three replicates), with no sodium perchlorate in the control or dark-treated salami replicates. The magnitude of

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Table 6. Residues of Sodium Chlorate and Sodium Perchlorate in Chlorine Dioxide Treated Salami

				analyte	
treatment	replicate	salami set		chlorate[a] (ng/g)	perchlorate[b] (ng/g)
no ClO_2–dark	day 3	A		53.0	<LOQ
		B		33.1	<LOQ
			replicate mean	43.1	<LOQ
	day 4	A		50.0	<LOQ
		B		31.3	<LOQ
			replicate mean	40.7	<LOQ
	day 5	A		36.8	<LOQ
		B		31.0	<LOQ
			replicate mean	33.9	<LOQ
			treatment mean	**39.2 ± 4.8**	**<LOQ**
ClO_2–light	day 3	A		127.4	4.6
		B		159.2	4.7
			replicate mean	143.3	4.7
	day 4	A		123.2	<LOQ
		B		150.5	<LOQ
			replicate mean	136.9	<LOQ
	day 5	A		241.9	4.4
		B		299.0	4.6
			replicate mean	270.5	4.5
			treatment mean	**183.6 ± 75.4**	**3.7 ± 1.5**[c]
ClO_2–dark	day 3	A		135.3	<LOQ
		B		128.2	<LOQ
			replicate mean	131.7	<LOQ
	day 4	A		131.5	<LOQ
		B		182.9	<LOQ
			replicate mean	157.2	<LOQ
	day 5	A		121.2	<LOQ
		B		95.2	<LOQ
			replicate mean	108.2	<LOQ
			treatment mean	**128.0 ± 31.2**	**<LOQ**

[a]Data are expressed on a sodium chlorate equivalent basis, LOQ = 5 ng/g. [b]Data are expressed on a sodium perchlorate equivalent basis, LOQ = 4 ng/g. [c]Mean calculated by including the <LOQ replicate at the method half the method LOQ for sodium perchlorate (2 ng/g).

Table 7. Recoveries of Sodium Chlorate and Sodium Perchlorate from Fortified Salami

		analyte					
		sodium chlorate[a]				sodium perchlorate[b]	
		uncorrected		corrected[c]			
replicate	sample	ng/g	%	ng/g	%	ng/g	%
day 3	A	114.2	285.5	75.0	187.5	17.9	89.5
	B	87.1	217.6	47.9	119.6	15.3	76.5
day 4	A	76.1	190.3	36.9	92.3	13.6	68.0
	B	90.3	225.8	51.1	127.8	14.0	70.0
day 5	A	87.2	217.9	48.0	119.9	14.1	70.5
	B	83.5	208.8	44.6	111.4	13.5	67.3
		89.7 ± 12.9	**224.3 ± 32.3**	**50.6 ± 12.9**	**126.4 ± 32.3**	**14.7 ± 1.7**	**73.6 ± 8.4**

[a]Data are expressed on a sodium chlorate equivalent basis. [b]Data are expressed on a sodium perchlorate equivalent basis. [c]Sodium chlorate data corrected for the average content of sodium chlorate in negative control (blank) samples (39.2 ng/g).

the sodium chlorate residues in this study was roughly one-tenth that measured in rind + edible flesh of cantaloupe of Smith et al.[33] The difference in magnitude of residue between the two studies is likely related to both the target chlorine dioxide dose and perhaps the composition of salami (high protein and fat) compared to cantaloupe rind (highly lignified carbohydrate content). For this study, a target concentration of 55 mg of chlorine dioxide per kilogram of salami was generated, whereas for studies with cantaloupe, a target of 100 mg of gas per kilogram of melon was used. Chlorate accumulation on cantaloupe rind relative to the surface of salami suggests that chlorine dioxide had a greater propensity to disproportionate on cantaloupe.

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Recovery values for sodium chlorate and sodium perchlorate fortified into blank salami are shown in Table 7. As previously mentioned, untreated salami contained variable quantities of sodium chlorate; therefore, the recovery values shown in Table 7 are high. For example, recovery of sodium chlorate was 224% in salami puree fortified with 40 ng/g sodium chlorate. After correction for the sodium chlorate content of the untreated salami, the average recovery of sodium chlorate in the fortified samples was 126.4 ± 32.3%. Both the recovery and the variance around the recovery value are too high for regulatory assays, but for research purposes are sufficient to establish the magnitude of the chlorate residue present in the fumigated salami. Given the high recoveries recorded in this study, the reported sodium chlorate residue data would represent high estimates, or worst-case residues, of sodium chlorate under the chlorine dioxide exposure conditions used in this study.

The presence of sodium chlorate in untreated salami was unexpected, but on reflection perhaps not surprising. For example, in a recent survey[43] of plant-based food samples, chlorate residues were quantified in nearly one-fourth (24.5%) of the 1087 samples tested; concentrations of chlorate ranged from 10 to 2700 ng/g of vegetable matter. Furthermore, Kettlitz et al.[44] reported that the incidence of chlorate in pork was 64% (n = 14) with concentrations of chlorate ranging from <10 to 46 ng/g. The authors of the CVUA report[43] did not attribute specific sources of chlorate to the measured contamination. However, Gil et al.[45] provided data implicating the use of hypochlorite-based rinse waters in chlorate contamination of food. For ready-to-eat meats, and perhaps some of the contaminated vegetables measured by the CVUA study, one source of chlorate contamination could be hypochlorite-based sanitizing solutions. Sodium hypochlorite solutions can be unstable, with a major degradation product being sodium chlorate. In fact, the formation of gram quantities of sodium chlorate per liter (i.e., parts per thousand concentrations) in hypochlorite solution has been measured in controlled studies[46] and in surveys of stored industrial bleach solutions.[47] If a contaminated bleach solution containing parts per thousands of chlorate was used for sanitation of meat-processing equipment or for a salami rinse prior to storage or curing, part per billion chlorate residues might result. Currently, a default maximum residue (MRL) level of 0.01 mg/kg exists in the European Union,[48] but the European Food Safety Authority suggested that a hypothetical MRL of 0.7 mg/kg, consistent with the World Health Organization MRL of 0.7 mg/L in drinking water, would have minimal impacts on acute and chronic human chlorate exposures.[48]

Chlorate residues arising from chlorine dioxide sanitation have been previously measured in chlorine dioxide treated tomatoes and cantaloupe[30,32,33] and are thought to originate from chlorine dioxide disproportionation reactions.[34,35] Protection of chlorine dioxide from light during the sanitation process reduces or eliminates the formation of chlorate.[33] ^{36}Cl-labeled chlorite residues, however, were not previously detected[30,32] in cantaloupe or tomato puree subsequent to chlorine dioxide sanitation. The lack of chlorite residue in plant material is likely due its reactivity with plant components,[33] the high moisture content, and the relative acidities of tomato (pH 4.2−4.9)[49] and cantaloupe flesh (pH 6.1−7.1).[49] Smith et al.[30] demonstrated the instability of >100 μg/g of sodium ^{36}Cl-chlorite in tomato puree with spiking experiments; ^{36}Cl-chlorite residues in tomato puree quantitatively degraded with chloride ion as the major product and small amounts of chlorate forming. Thus, the detection of ^{36}Cl-chlorite, even at trace levels, after treatment of salami with $^{36}ClO_2$ gas was unexpected. Of note, however, ^{36}Cl-chlorite was not detected in any of the six aliquots measured in experiment A and in only three of six aliquots measured in experiment B.

When performed in the absence of light, sanitation of ready-to-eat salami with chlorine dioxide (6 h exposure) did not cause the formation of measurable (4 ng/g) sodium perchlorate residues. In contrast, sodium chlorate residues were present in both control (39.2 ± 4.8 ng/g) and chlorine dioxide treated (128 ± 31.2 ng/g) salami. If sanitation occurred under conditions of illumination, trace levels (3.7 ± 1.5 ng/g) of perchlorate were formed along with greater quantities of sodium chlorate (183.6 ± 75.4 ng/g). When salami was present, chlorine dioxide did not accumulate in treatment chambers, whereas when salami was absent, chlorine dioxide release from slow-release media and accumulation in treatment chambers were nearly identical to theoretical releases and accumulation. Collectively, these data suggest that chlorine dioxide is chemically reduced quickly on salami surfaces and that slow-release formulations could be used for ready-to-eat sanitation provided that chlorate residues fall within limits set by regulatory organizations.

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Notes

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REFERENCES

(1) Gómez-López, V. M.; Rajkovic, A.; Ragaert, P.; Smigic, N.; Devlieghere, F. Chlorine dioxide for minimally processed produce preservation: a review. *Trends Food Sci. Technol.* **2009**, *20*, 17−26.

(2) Jin-hua, D. U.; Mao-run, F. U.; Miao-miao, L. I.; Wei, X. I. A. Effects of chlorine dioxide gas on postharvest physiology and storage quality of green bell pepper (*Capsicum frutescens* L. var Longrum). *Agric. Sci. China* **2007**, *6*, 214−219.

(3) Mahovic, M. J.; Tenney, J. D.; Bartz, J. A. Applications of chlorine dioxide gas for control of bacterial soft rot in tomatoes. *Plant Dis.* **2007**, *91*, 1316−1320.

(4) Trinetta, V.; Linton, R. H.; Morgan, M. T. Use of chlorine dioxide gas for the postharvest control of *Alternaria alternata* and *Stemphylium vesicarium* on Roma tomatoes. *J. Sci. Food Agric.* **2013**, *93*, 3330−3333.

(5) Southwell, K. L. Chlorine dioxide: a treatment for mold in libraries. *Abbey Newsl.* **2003**, *26*, 98−99.

(6) Nam, H.; Seo, H.-S.; Bang, J.; Kim, H.; Beuchat, L. R.; Ryu, J.-H. Efficacy of gaseous chlorine dioxide in inactivating *Bacillus cereus* spores attached to and in a biofilm on stainless steel. *Int. J. Food Microbiol.* **2014**, *188*, 122−127.

(7) Wang, T.; Qi, J.; Wu, J.; Hao, L.; Yi, Y.; Lin, S.; Zhang, Z. Response surface modeling for the inactivation of *Bacillus subtilis* supsp. *niger* spores by chlorine dioxide gas in an enclosed space. *J. Air Waste Manage. Assoc.* **2016**, *66*, 508−517.

(8) Sy, K. V.; McWatters, K. H.; Beuchat, L. R. Efficacy of gaseous chlorine dioxide as a sanitizer for killing *Salmonella*, yeasts, and molds

DOI: 10.1021/acs.jafc.6b04011
J. Agric. Food Chem. 2016, 64, 8454−8462

on blueberries, strawberries, and raspberries. *J. Food Prot.* **2005**, *68*, 1165−1175.
(9) Wu, V. C. H.; Rioux, A. A simple instrument-free gaseous chlorine dioxide method for microbial decontamination of potatoes during storage. *Food Microbiol.* **2010**, *27*, 179−184.
(10) Simonet, J.; Gantzer, C. Degradation of the *Poliovirus 1* genome by chlorine dioxide. *J. Appl. Microbiol.* **2006**, *100*, 862−870.
(11) Yeap, J. W.; Kaur, S.; Lou, F.; DiCaprio, E.; Morgan, M.; Linton, R.; Li, J. Inactivation kinetics and mechanism of a human norovirus surrogate on stainless steel coupons via chlorine dioxide gas. *Appl. Environ. Microbiol.* **2016**, *82*, 116−123.
(12) Han, Y.; Guentert, A. M.; Smith, R. S.; Linton, R. H.; Nelson, P. E. Efficacy of chlorine dioxide gas as a sanitizer for tanks used for aseptic juice storage. *Food Microbiol.* **1999**, *16*, 53−61.
(13) U.S. EPA. Reregistration eligibility decision (RED) for chlorine dioxide and sodium chlorite (Case 4023). EPA 738-R-06-007, 2006; https://archive.epa.gov/pesticides/reregistration/web/pdf/chlorine_dioxide_red.pdf (accessed Aug 19, 2016).
(14) Kang, J. H.; Park, S. M.; Kim, H. G.; Son, H. J.; Song, K. J.; Cho, M.; Kim, J. R.; Lee, J. Y.; Song, K. B. Effects of combined chlorine dioxide gas treatment using low-concentration generating sticks on the microbiological safety and quality of paprika during storage. *Han'guk Sikp'um Yongyang Kwahak Hoechi* **2016**, *45*, 619−624.
(15) Hsu, C.-S.; Huang, D.-J. Disinfection efficiency of chlorine dioxide gas in student cafeterias in Taiwan. *J. Air Waste Manage. Assoc.* **2013**, *63*, 796−805.
(16) Ogata, N.; Shibata, T. Protective effect of low-concentration chlorine dioxide gas against influenza A virus infection. *J. Gen. Virol.* **2008**, *89*, 60−67.
(17) Ogata, N.; Sakasegawa, M.; Miura, T.; Shibata, T.; Takigawa, Y.; Taura, K.; Taguchi, K.; Matsubara, K.; Nakahara, K.; Kato, D.; Sogawa, K.; Oka, H. Inactivation of airborne bacteria and viruses using extremely low concentrations of chlorine dioxide gas. *Pharmacology* **2016**, *97*, 301−306.
(18) CFR. Code of Federal Regulations Title 21, 21CFR173.300; http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=173.300 (accessed May 25, 2016).
(19) EPA. Notice of Pesticide Registration, 79814-5; https://iaspub.epa.gov/apex/pesticides/f?p=PPLS:102:::NO::P102_REG_NUM:79814-5 (accessed June 1, 2016).
(20) Abdel-Rahman, M. S.; Couri, D.; Jones, J. D. Chlorine dioxide metabolism in rat. *J. Environ. Pathol. Toxicol.* **1980**, *31*, 431−430.
(21) Abdel-Rahman, M. S.; Couri, D.; Bull, R. J. Metabolism and pharmacokinetics of alternate drinking water disinfectants. *Environ. Health Perspect.* **1982**, *46*, 19−23.
(22) Abdel-Rahman, M. S.; Couri, D.; Bull, R. J. The kinetics of chlorite and chlorate in the rat. *J. Am. Coll. Toxicol.* **1984**, *3*, 261−267.
(23) Scatina, J.; Abdel-Rhaman, M. S.; Gerges, S. E.; Alliger, H. Pharmacokinetics of Alcide©, a germicidal compound in rat. *J. Appl. Toxicol.* **1983**, *3*, 150−153.
(24) Scatina, J.; Abdel-Rhaman, M. S.; Gerges, S. E.; Khan, M. Y.; Gona, O. Pharmacodynamics of alcide, a new antimicrobial compound, in rat and rabbit. *Fundam. Appl. Toxicol.* **1984**, *4*, 479−484.
(25) Hakk, H.; Smith, D. J.; Shappell, N. W. Tissue residues, metabolism, and excretion of radiolabeled sodium chlorate (Na[^{36}Cl]-ClO_3) in rats. *J. Agric. Food Chem.* **2007**, *55*, 2034−2042.
(26) Smith, D. J.; Anderson, R. C.; Ellig, D. A.; Larsen, G. Tissue distribution, elimination, and metabolism of dietary sodium [^{36}Cl]-chlorate in beef cattle. *J. Agric. Food Chem.* **2005**, *53*, 4272−4280.
(27) Smith, D. J.; Oliver, C. E.; Caton, J. S.; Anderson, R. C. Effect of sodium [^{36}Cl]chlorate dose on total radioactive residues and residues of parent chlorate in beef cattle. *J. Agric. Food Chem.* **2005**, *53*, 7352−7360.
(28) Smith, D. J.; Anderson, R. C.; Huwe, J. K. Effect of sodium [^{36}Cl]chlorate dose on total radioactive residues and residues of parent chlorate in growing swine. *J. Agric. Food Chem.* **2006**, *54*, 8648−8653.
(29) Smith, D. J.; Byrd, J. A.; Anderson, R. C. Total radioactive residues and residues of [^{36}Cl]chlorate in market size broilers. *J. Agric. Food Chem.* **2007**, *55*, 5898−5903.
(30) Smith, D. J.; Ernst, W.; Giddings, J. M. Distribution and chemical fate of ^{36}Cl-chlorine dioxide gas during the fumigation of tomatoes and cantaloupe. *J. Agric. Food Chem.* **2014**, *62*, 11756−11766.
(31) Smith, D. J.; Oliver, C. E.; Shelver, W. L.; Caesar, T.-C.; Anderson, R. C. Chlorate metabolism in pure cultures of *Escherischia coli* O157:H7 pretreated with either nitrate or chlorate. *J. Agric. Food Chem.* **2009**, *57*, 10216−10224.
(32) Kaur, S.; Smith, D. J.; Morgan, M. T. Chloroxyanion residue quantification in cantaloupes treated with chlorine dioxide gas. *J. Food Prot.* **2015**, *78*, 1708−1718.
(33) Smith, D. J.; Ernst, W.; Herges, G. R. Chloroxyanion residues in cantaloupe and tomatoes after chlorine dioxide gas sanitation. *J. Agric. Food Chem.* **2015**, *63*, 9640−9649.
(34) Nabar, G. M.; Ramachandran, C. R.; Shenai, V. A. Studies in sodium chlorite: Part II—The mechanism of photodecomposition of sodium chlorite. *Ind. J. Technol.* **1964**, *2*, 11−14.
(35) Vel Leitner, N. K.; De Laat, J.; Dore, M. Photodecomposition of chlorine dioxide and chlorite by U.V.-irradiation—Part I. Photo-products. *Water Res.* **1992**, *26*, 1655−1664.
(36) Sofos, J. N.; Geornaras, I. Overview of current meat hygiene and safety risks and summary of recent studies on biofilms, and control of *Escherichia coli* O157:H7 in nonintact, and *Listeria monocytogenes* in ready-to-eat, meat products. *Meat Sci.* **2010**, *86*, 2−14.
(37) Crum, N. F. Update on *Listeria monocytogenes* infection. *Curr. Gastroenterol. Rep.* **2002**, *4*, 287−296.
(38) Shin, J.; Harte, B.; Selke, S.; Lee, Y. Use of a controlled chlorine dioxide (ClO_2) release system in combination with modified atmosphere packaging (MAP) to control growth of pathogens. *J. Food Qual.* **2011**, *34*, 220−228.
(39) Du, J.; Han, Y.; Linton, R. H. Inactivation by chlorine dioxide gas (ClO_2) of *Listeria monocytogenes* spotted onto different apple surfaces. *Food Microbiol.* **2002**, *19*, 481−490.
(40) Mahmoud, B. S. M.; Vaidya, N. A.; Corvalan, C. M.; Linton, R. H. Inactivation kinetics of inoculated *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella* Poona on whole cantaloupe by chlorine dioxide gas. *Food Microbiol.* **2008**, *25*, 857−865.
(41) Smith, D. J.; Taylor, J. B. Chlorate analyses in matrices of animal origin. *J. Agric. Food Chem.* **2011**, *59*, 1598−1606.
(42) Ghanbari, H. A.; Wheeler, W. B.; Kirk, J. R. Reactions of aqueous chlorine and chlorine dioxide with lipids: Chlorine incorporation. *J. Food Sci.* **1982**, *47*, 482−485.
(43) CVUA. Chlorate residues in plant-based food: Origin unknown. Chemisches und Veterinäruntersuchungsamt Stuttgart; http://www.cvuas.de/pub/beitrag.asp?subid=1&ID=1854&Pdf=No (accessed Aug 22, 2016).
(44) Kettlitz, B.; Kemendi, G.; Thorgrimsson, N.; Cattoor, N.; Verzegnassi, L.; Le Bail-Collet, Y.; Maphosa, F.; Perrichet, A.; Christall, B.; Stadler, R. Why chlorate occurs in potable water and processed foods: a critical assessment and challenges faced by the food industry. *Food Addit. Contam., Part A* **2016**, *33*, 968−982.
(45) Gil, M. I.; Marín, A.; Andujar, S.; Allende, A. Should chlorate residues be of concern in fresh-cut salads? *Food Control* **2016**, *60*, 416−421.
(46) Stanford, B. D.; Pisarenko, A. N.; Snyder, S. A.; Gordon, G. Perchlorate, bromate, and chlorate in hypochlorite solutions: guidelines for utilities. *J. Am. Water Works Assoc.* **2011**, *103*, 1−13.
(47) Asami, M.; Kosaka, K.; Kunikane, S. Bromate, chlorate, chlorite, and perchlorate in sodium hypochlorite solution used in water supply. *Aqua* **2009**, *58*, 107−115.
(48) EFSA.. Risks for public health related to the presence of chlorate in food. EFSA panel on contaminants in the food chain. *EFSA J.* **2015**, *13*, 4135.
(49) U.S. FDA. Bad Bug Book. Foodborne pathogenic microorganisms and natural toxins handbook, pH values of various foods; http://www.fda.gov/Food/FoodborneIllnessContaminants/CausesOfIllnessBadBugBook/ucm122561.htm (accessed August 22, 2016).

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