The Effect of Controlled-release Chlorine Dioxide on the Preservation of Grapefruit

Xiuxiu Sun

U.S. Horticultural Research Laboratory, USDA, ARS, 2001 S. Rock Road, Fort Pierce, FL 34945; and Indian River Research and Education Center, University of Florida, 2199 S. Rock Rd Ft. Pierce, FL 34845

Elizabeth Baldwin, Chris Ference, Jan Narciso, and Anne Plotto

U.S. Horticultural Research Laboratory, USDA, ARS, 2001 S. Rock Road, Fort Pierce, FL 34945

Mark Ritenour

34945 Indian River Research and Education Center, University of Florida, 2199 S. Rock Road, Ft. Pierce, FL 34845

Ken Harrison and Dave Gangemi

Worrell Water Technologies, LLC, 4004 Hunterstand Court, Charlottesville, VA 22911

Jinhe Bai¹

U.S. Horticultural Research Laboratory, USDA, ARS, 2001 S. Rock Road, Fort Pierce, FL 34945

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Abstract. The effect of controlled-release chlorine dioxide (ClO₂) gas on the safety and quality of grapefruit was studied. The experiments were run under controlled chamber systems with inoculated fruit, and in boxed fruit under commercial conditions. For the inoculation test, fruit artificially inoculated with either *Escherichia coli* or *Penicillium digitatum*, or naturally inoculated *Xanthomonas citri* ssp. *citri* (*Xcc*) (fruits with citrus canker lesions), were incubated in a chamber containing a dose equivalent to 0–60 mg·L⁻¹ of pure ClO₂ as an antimicrobial agent. After 24 hours, the microbial population on treated grapefruit was significantly reduced compared with that of control fruit: a dosage of 5 mg·L⁻¹ completely inhibit the growth of *E. coli* and *P. digitatum*, but a dosage of 60 mg·L⁻¹ was needed to completely kill *Xcc*. For the simulated commercial experiment, fruit were harvested in late Oct. 2015 passed through a commercial packing line, and packed in 29 L citrus boxes. ClO₂ packets were attached to the top lids with the following five treatments: fast-release, slow-release, slow/fast-release combination (each containing 14.5 mg·L⁻¹ of pure ClO₂), double dose fast-release (containing 29 mg·L⁻¹ of ClO₂), and control. After 6 weeks of storage at 10 °C (to simulate storage and transportation) + 1 week of storage at 20 °C (to simulate retail marketing), the fruit quality was evaluated. The slow-release treatment at standard dose exhibited the best antimicrobial activity, reducing total aerobic bacterial count and yeast/mold count by 0.95 and 0.94 log colony-forming units (cfu)/g of fruit, respectively, and maintained the best visual, sensory, and overall quality. However, the higher dosage treatments resulted in phytotoxicity as evidenced by peel browning.

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¹Corresponding author. E-mail: jinhe.bai@ars.usda.gov.

Fresh produce is the main source of most foodborne illnesses in the United States, reported to the Centers for Disease Control and Prevention (CDC) (Herman et al., 2015). Between 2011 and 2013 the U.S. CDC reported 140 foodborne disease outbreaks associated with consumption of various contaminated foods. Many outbreaks of human gastroenteritis have been linked to the consumption of contaminated fresh vegetables and fruits (Garner and Kathariou, 2016). Enterotoxigenic *E. coli*, specifically serotype O157:H7, is the most common cause of diarrhea (Trainor et al., 2016), and consumption of *E. coli* O157:H7 contaminated cantaloupes caused an outbreak of gastroenteritis (Denis et al., 2016). Besides implementation

of food safety plans with sound prerequisite programs and appropriate preventive controls for identified hazard to reduce the risk of foodborne infections, it is important to develop improved decontamination technologies for both food contact surfaces and the food itself. Furthermore, an estimated 24% of all fresh produce globally is lost postharvest due to microbial spoilage (Mahajan et al., 2014). Improved decontamination technology could also reduce surface microbial populations and subsequent postharvest spoilage.

Evidence from a large number of epidemiological studies, both in vitro and in vivo, has shown that consumption of citrus has many health benefits (Vanamala et al., 2006). Grapefruit (*Citrus ×paradisi*) is a subtropical citrus fruit known for its sour to semisweet property (Murphy et al., 2014). Grapefruit juice contains high amounts of the flavanones naringenin and hesperetin, which exhibit estrogenic, anticarcinogenic, and antioxidative properties (Murphy et al., 2014; Reddy et al., 2007). However, grapefruit are susceptible to attack by the fungi *P. digitatum* Sacc and *Penicillium italicum* Wehmer, which cause green and blue molds, respectively, and account for most of the post-harvest losses of citrus fruits worldwide (Louw and Korsten, 2015). Although not causing fruit decay, citrus canker, caused by the pathogenic bacterium *X. citri* ssp. *citri* (*Xcc*), is another serious disease affecting grapefruit production in several tropical and subtropical areas of the world (Bock et al., 2011).

ClO₂ is a water-soluble oxidizer with an oxidation capacity 2.5 times greater than that of free chlorine (Sun et al., 2014; Wang et al., 2014). It has been used in postharvest processes to reduce microbial populations on fruits and vegetables in both aqueous and gaseous forms (Gomez-Lopez et al., 2009; Sun et al., 2014; Sy et al., 2005a). However, the instability of aqueous ClO₂ and the equipment requirements for on-site production of gaseous ClO₂ have limited its application (Chomkitichai et al., 2014). Curoxin® ClO₂ (9.5% w/w ClO₂ slurry, Worrell Water Technologies, Charlottesville, VA) is stable, ready-to-use, and available in an easily transportable form. Due to its proprietary microencapsulation, the release of ClO₂ gas can be regulated, providing sustained delivery of ClO₂ for improved disinfection and reduced spoilage.

Nevertheless, to the best of our knowledge, no information related to the effect of controlled-release ClO₂ on the safety and quality of grapefruit has been conducted to date. The objective of this research was to evaluate an advanced form of ClO₂ (Curoxin® ClO₂) delivered in sealed pouches with selectable release rates for use in eliminating pathogens and extending shelf life of grapefruit.

Materials and Methods

Fruits. Grapefruit (*Citrus ×paradisi*, var. Ruby Red), grown in Vero Beach, FL, were used for all experiments. For the chamber inoculation experiment, defect free, and diseased fruits with canker lesions, 20 fruit per treatment, were obtained from an experimental

block on Nov. 23, 2015, and stored at 10 °C for 1–7 d before fruit inoculation and incubation. To simulated commercial storage/transportation/marketing conditions, fruit harvested from a commercial field in Oct. 2015 were passed through a commercial packing line and 56 fruits (average fruit weight 260 g and diameter 85 mm) were packed in each of 15, 29-L commercial perforated boxes that were then stored at 10 °C for 3 d before applying ClO₂ treatments.

Chamber test with microbial inoculated fruit. Strains of *E. coli* wild type and *P. digitatum* wild type were isolated from citrus fruit surfaces (Narciso et al., 2012) and stored at –80 °C on EC agar (ECA) made by EC Broth (Oxoid, UK) with 1.5% agar, and on potato dextrose agar (PDA, BD Difco, Sparks, MD) plugs in 10% glycerol (cryoprotectant), respectively. The ECA plugs were recultured on ECA at 35 °C for a week and the bacteria were checked on Levine eosin methylene blue (EMB) agar. The PDA plugs were recultured on PDA at 25 °C for a week and identified by spore production. For inoculum preparation, the *E. coli* and *P. digitatum* cells and spores were scraped from 4 d and 7 d old cultures on ECA and PDA media, respectively, using a sterile loop with a drop of 0.1% Tween-20 (Fisher Scientific Inc., Pittsburgh, PA), to aid in even spore suspension. Cells/spores were suspended into 2 L of sterile distilled water at room temperature for inoculation at populations of 7.29 and 7.56 log/mL for *E. coli* and *P. digitatum*, respectively.

The fruit were completely submerged in the inoculum for 30 s and then placed onto an open shelf and allowed to dry for 2 h at 20 ± 2 °C in a biosafety flow hood.

Individual inoculated fruit were incubated in a glass chamber containing a dose equivalent to 5, 10, or 15 mg·L⁻¹ of gaseous ClO₂ at 20 °C for 24 h, and microbial survival was detected. For the *Xcc* test, naturally infected fruit with visible canker lesions were incubated under the same conditions as above except the ClO₂ dosage was increased to 0, 20, 40, or 60 mg·L⁻¹ based on a preliminary experiment. All treatments included three replicates.

Boxed fruit under simulated commercial conditions. The ClO₂ packets were prepared using heat sealing plastic films containing 0.5 g Curoxin® ClO₂ slurry (9.5% a.i.). The slow and fast releases were regulated by selecting films with different ClO₂ permeance. The effective surface area was 6 cm². The packets were attached on the top lids of the boxes with double-sided tape and the following five treatments were applied: single dose fast-release (two packets, F), single dose slow-release (two packets, S), combination of single dose slow and single dose fast-release (one packet for each type, FS), double dose fast-release (four packets, FF), and non-ClO₂ control (C). Each treatment contained three replicates, and each replicate contained 56 fruits in one box. After 42 d storage at 10 °C (to simulate storage and transportation) + 7 d storage at 20 °C (to simulate retail market), each box (replicate) was unpacked, and the fruit were evaluated for visual quality, peel disorders (browning),

stem-end rot, sensory quality, and microbial populations.

Microbiological analysis. Under sterile conditions, fruit samples (one from each replicate) were agitated for 1 h in a sterilized sampling bag (Fisherbrand, Fisher Scientific, Pittsburgh, PA) along with 99 mL of sterile potassium phosphate buffer (0.01 M, pH 7.2) on an orbital shaker (Innova 2100; New Brunswick Scientific, New Brunswick, NJ). A dilution series were prepared and cultured on different media for microbial counts. ECA media was used for enumerating *E. coli*, PDA for enumerating yeast/mold, nutrient agar (NA, BD Difco, Sparks, MD) for enumerating *Xcc*, and plate count agar (PCA, BD Difco, Sparks, MD) for enumerating total bacteria count using an Eddy Jet Spiral Plater (Neutec Group Inc., Farmingdale, NY). ECA, PDA, NA, and PCA were incubated at 35 °C for 24 h, 25 °C for 3 d, 35 °C for 24 h, and 25 °C for 2 d, respectively (Ruparelia et al., 2008; Sun et al., 2014), and the results were read on a ProtoCOL colony counter (Synoptics, Ltd., Cambridge, UK). Fungal and bacterial populations were expressed as log cfu/gram of fruit. All tests were run in triplicate.

Visual quality evaluation. The overall visual quality, peel browning, and incidence of stem-end rot were evaluated for individual fruit in the commercial citrus box. Fruit visual quality was subjectively scored using a 1–9 scale (9 = excellent, 7 = good, 5 = fair, 3 = poor, and 1 = completely deteriorated) (Bai et al., 2003). A score of 5 was considered the threshold level for marketability, and 3 for edibility. All criteria were then normalized to a scale of 0–1 (worst–best) by feature scaling (Aksoy and Haralick, 2001) for average fruit quality in each treatment. Percentage of peel browning and incidence of stem-end rot were calculated by the total number of fruit exhibiting peel browning or stem-end rot symptoms. Fifty six fruit were evaluated individually per replicate and the percentage was used for data analysis. Every treatment includes three replicates.

Sensory evaluation. Sensory evaluation was carried out with fresh-cut grapefruit from the commercial citrus box by a panel of 10 staff members using a 1–5 index scale (1 = none and 5 = strong) testing for moldy off-flavor, chemical or other off-flavor, juiciness, and overall quality (Bai et al., 2011). All panel members were familiar with sensory evaluation of grapefruit and other fruits. Every panel member was presented with two 3-digit coded samples, one for each of slow-release ClO₂-treated and untreated control.

Weight loss. To determine weight loss, fruit in the commercial citrus box were weighed at the beginning and during storage. Weight loss was expressed as the percentage loss of the initial total weight.

Statistical analyses. All experiments were replicated at least three times. Data were analyzed using analysis of variance with SPSS, version 17.0 software (Experian QAS, Boston, MA). Mean separation was determined by Duncan's multiple range test. Significance was defined at $P < 0.05$.

Results and Discussion

In the chamber experiment, microbial growth was significantly inhibited compared with the control, and the lowest concentration of ClO₂ (5 mg·L⁻¹) completely inhibited the growth of *E. coli* and *P. digitatum*. Dosages of 20 mg·L⁻¹ and 40 mg·L⁻¹ reduced the population of *Xcc* by 2.44 and 6.45 logs, respectively, compared with the control and the dosage of 60 mg·L⁻¹ completely inhibited the growth of *Xcc* (Table 1). ClO₂ has broad antimicrobial efficiency against many pathogenic and spoilage microorganisms, such as *E. coli* O157:H7, *Salmonella* sp., *Penicillium expansum*, and *Xanthomonas campestris* pv. *campestris* (Krauthausen et al., 2011; Mahmoud et al., 2008; Okull et al., 2006). It reduces microorganism populations by oxidation, mainly through the one-electron transfer mechanism, in which the compound itself is reduced to chlorite (Netramai et al., 2012). Treatment with 200 µg·mL⁻¹ ClO₂ for 10 min showed greater lethal activity than NaOCl applied at the same concentration for the same duration against *E. coli* O157:H7 in a biofilm (Bang et al., 2014). The mode of action of ClO₂ on *E. coli* is thought to be due to the loss of potassium membrane permeability control, along with nonspecific oxidative damage to the outer membrane, all leading to the destruction of the transmembrane ionic gradient (Berg et al., 1986). Inoculum of *P. digitatum* in a commercial soak tank was reduced by maintaining 5–10 mg·L⁻¹ of available ClO₂ in the tank (Brown and Wardowski, 1984). A concentration of 3 mg·L⁻¹ ClO₂ was effective against *X. campestris* pv. *campestris* in brassica transplants (Krauthausen et al., 2011). These results indicate that ClO₂ can be used as an antimicrobial agent against certain microorganisms.

In the simulated commercial experiment, the slow-release treatment at standard dose reduced yeast/mold, and total bacterial count compared with the control by 0.94 and 0.95 logs cfu/g, respectively (Table 2). Similar results have been reported with treatments of 60 and 80 mg·L⁻¹ of aqueous ClO₂ in mulberry fruit, where the population of aerobic

Table 1. Effects of ClO₂ on microbial populations on individual grapefruit after inoculation with either *Escherichia coli* or *Penicillium digitatum*, and then incubation at 20 °C for 24 h in individual chambers. Populations of *Xanthomonas citri* (*Xcc*) were also evaluated but came from natural infection in the field. Microbial count is expressed as log colony-forming units (cfu)/g.

ClO ₂ concn (mg·L ⁻¹)	<i>E. coli</i> (log cfu/g)	<i>P. digitatum</i> (log cfu/g)	<i>Xcc</i> (log cfu/g)
0	5.31 a ²	5.30 a	7.92 a
5	0 b	0 b	
10	0 b	0 b	
15	0 b	0 b	
20			5.48 b
40			1.47 c
60			0 d

²Mean values followed by different letters within a column indicate significant differences using Duncan test ($P < 0.05$).

Table 2. Effects of ClO₂ on microbial populations on grapefruit held under commercial conditions (storage at 10 °C for 42 d + 20 °C for 7 d), expressed in log colony-forming units (cfu)/g.

Treatment	Yeast/mold (log cfu/g)	Total bacteria (log cfu/g)
C	5.90 a [*]	6.11 a
F	5.06 b	5.40 ab
S	4.96 b	5.16 b
FS	5.08 b	5.38 ab
FF	5.01 b	5.35 ab

C = control, F = single fast-release, S = single slow-release, FS = single fast/slow combination, and FF = double fast-release.

^{*}Mean values followed by different letters within a column indicate significant differences using Duncan test ($P < 0.05$).

mesophilic bacteria, aerobic psychrotrophic bacteria, lactic acid bacteria, and yeast and mold were significantly reduced (Chen et al., 2011). In addition, a gaseous treatment with 12.0 mg·L⁻¹ for 10 min, 7.2 mg·L⁻¹ for 20 min, or 4.8 mg·L⁻¹ for 30 min of ClO₂ at 21 °C and 90% to 95% relative humidity completely inactivated 8 log cfu/site of *E. coli* O157:H7 that had been initially inoculated on apple skin (Du et al., 2003). Treatments with 0.62 and 1.24 mg·L⁻¹ ClO₂ gas for 30 min at 22 °C and 90% to 95% relative humidity reduced *E. coli* O157:H7 × 3.03 and 6.45 log, respectively, on surface-injured green peppers (Han et al., 2000). Total aerobic bacteria, yeasts and molds on strawberries were significantly inhibited by 50 ppm of aqueous ClO₂ (Jin et al., 2007). It was concluded that ClO₂, both in gaseous and aqueous forms, was a promising antimicrobial agent for fresh fruits and vegetables.

Along with the control, fruit exposed to the “slow” ClO₂ treatment maintained relatively high overall visual quality, and were significantly better than fruit exposed to the double fast-release ClO₂ treatment (Fig. 1A). The percentage of marketable fruit (score ≥ 5) under “slow” ClO₂ treatment was 89.3%, which is higher than that of fast/slow combination (76.2%) or double fast-release (61.3%) treatment (Fig. 1B). There was high incidence of chemical burn in the double fast-release treatment, although this treatment showed the best control of stem-end rot, whereas the “slow” ClO₂ treatment was next most effective with no browning (Fig. 2). However, there was no significant chemical burn detected on fruit receiving the “slow” ClO₂ treatment.

Appearance is one of the most important factors influencing consumer acceptance of fruits and vegetables (Bai et al., 2011). Browning is generally produced by the oxidation of phenols into quinones that subsequently polymerize into brown pigments (melanins), a reaction catalyzed by polyphenol oxidase (Gomez-Lopez, 2002). Gaseous ClO₂ can immediately induce browning of cabbage (Sy et al., 2005b). The results in the present research suggest a similar effect of the ClO₂ resulting in browning. Diplodia stem-end rot of citrus fruits, caused by the fungus *Lasiodiplodia theobromae*, is a typical postharvest disease in Florida and other subtropical and

Fig. 1. Effects of ClO₂ on overall visual quality of grapefruit held under commercial conditions (storage at 10 °C for 42 d + 20 °C for 7 d). Fruit visual quality (A) was subjectively scored using a 1–9 scale (9 = excellent, 7 = good, 5 = fair, 3 = poor, and 1 = completely deteriorated). The average fruit quality in each treatment was normalized to a 0–1 scale. The percentages of marketable fruits (B, quality scale higher or equal to 5) was calculated. C = control, F = single fast-release, S = single slow-release, FS = single fast/slow combination, and FF = double fast-release. Bars with different letters within each number indicate significant differences using Duncan test ($P < 0.05$).

Fig. 2. Effects of ClO₂ on incidence stem-end rot (A) and phytotoxicity browning (B) visual quality of grapefruit held under commercial conditions (storage at 10 °C for 42 d + 20 °C for 7 d), expressed in percentage (%). C = control, F = single fast-release, S = single slow-release, FS = single fast/slow combination, and FF = double fast-release. Bars with different letters within stem-end rot or browning indicate significant differences using Duncan test ($P < 0.05$).

tropical regions (Zhao et al., 2015). ClO₂ gas has potential as a postinoculation treatment of tomatoes for prevention of bacterial soft rot (Mahovic et al., 2007). Phytotoxic bleaching caused by ClO₂ gas has been detected in lettuce leaves after 15-min exposure to 0.75 mg·L⁻¹ ClO₂ (Singh et al., 2002). This may be due to the oxidation of chlorophyll during longer exposure times at higher concentrations of ClO₂. No color change was observed when milder treatment conditions were applied (Gomez-Lopez et al., 2009; Singh et al., 2002). These results, which agree with our own, suggest that a low concentration of ClO₂ will likely not cause adverse effects to the appearance of fresh fruits and vegetables.

Since the “slow” ClO₂ treatment showed the most promising results for visual quality, marketability, and control of stem-end rot without phototoxicity, a sensory test was done to determine if this treatment caused adverse sensory effects.

Fruit exposed to the “slow” ClO₂ treatment demonstrated better sensory quality by maintaining significantly higher juiciness and overall quality than for control (Fig. 3). Lee et al. (2012) evaluated the effects of aqueous ClO₂ and ultraviolet-C on the sensory properties of Romaine lettuce and kale and found the treatment provided better scores than the control. In addition, fresh-cut lettuce washed in a 3 mg·L⁻¹ aqueous ClO₂ did not adversely

Fig. 3. Effects of ClO₂ on sensory quality of grapefruit held under commercial conditions (storage at 10 °C for 42 d + 20 °C for 7 d). A 1–5 index scale (1 = none and 5 = strong) was used for the sensory evaluation with descriptors of moldy off-flavor (moldy), chemical or other off-flavor (chemical), juiciness, and overall quality. C = control and S = single slow-release. Bars with different letters within a descriptor indicate significant differences using Duncan test ($P < 0.05$).

affect sensory quality (Lopez-Galvez et al., 2010). Similarly, aqueous ClO₂ treatment did not damage blueberries (Rodgers et al., 2004). ClO₂ treatments did not cause any significant weight loss in this research (data not shown).

In conclusion, ClO₂ could be used as a sanitizer on grapefruit to reduce surface microbial populations including *E. coli*, citrus canker bacterium (*Xcc*) and fungal pathogen *P. digitatum*. ClO₂ also reduced stem-end rot incidence on grapefruit. The findings in this study suggest that a slow-release ClO₂ treatment in active packaging could improve the microbial safety, quality, and taste of grapefruit while reducing decay during storage and without impairing the appearance. This information contributes to increasing knowledge regarding the impact of controlled-release ClO₂ on food safety and quality and its potential use at commercial level.

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