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
34945Indian River Research and Education Center, University of Florida, 2199 S. Rock Rd 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

Additional index words. antimicrobial activity, citrus canker, stem-end rot, decay, Escherichia coli

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 inhibited 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.

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 canta-loupe 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 flavonones 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 Konsten, 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 with 1 = poorest level for marketability, and 3 for edibility. All criteria were then normalized to a score of 0–10 (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 analysis. 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 bacteria and fungal populations were significantly reduced (Krauthausen et al., 2011).

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).

<table>
<thead>
<tr>
<th>ClO2 concen (mg·L−1)</th>
<th>E. coli (log cfu/g)</th>
<th>P. digitatum (log cfu/g)</th>
<th>Xcc (log cfu/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5.31±a</td>
<td>5.30±a</td>
<td>7.92±a</td>
</tr>
<tr>
<td>5</td>
<td>10.0±b</td>
<td>10.0±b</td>
<td>0.5±c</td>
</tr>
<tr>
<td>15</td>
<td>0.5±c</td>
<td>0.5±c</td>
<td>0.5±c</td>
</tr>
<tr>
<td>20</td>
<td>5.48±b</td>
<td>5.48±b</td>
<td>0.5±c</td>
</tr>
<tr>
<td>40</td>
<td>1.47±c</td>
<td>1.47±c</td>
<td>0.5±c</td>
</tr>
<tr>
<td>60</td>
<td>0.2±d</td>
<td>0.2±d</td>
<td>0.5±c</td>
</tr>
</tbody>
</table>

*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 (70.2%) 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 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., 2012). 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 3 mg L−1 aqueous ClO2 did not adversely affect the appearance of fresh fruits and vegetables.
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 \textit{E. coli}, citrus canker bacterium \textit{(Xcc)} and fungal pathogen \textit{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 grapefruits 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.

**Literature Cited**


Bode, W. 1984. Effects of \textit{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).


solution and on apples, lettuce, strawberries, and cantaloupe. J. Food Prot. 67:721–731.