



Effect of controlled-release chlorine dioxide on the quality and safety of cherry/grape tomatoes



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ABSTRACT

The effect of controlled-release chlorine dioxide (ClO₂) gas on the food safety and quality of cherry/grape tomatoes was investigated. Whole grape tomatoes artificially inoculated with either *Escherichia coli* or *Alternaria alternata*, or whole cherry tomatoes inoculated with *Salmonella enterica* Newport, were packed in 1-lb clamshells, and stored at 20 °C for 14 days. ClO₂ pouches were attached under the lids with the following four dosages/release rates: single dose slow-release (S), single dose fast-release (F), fast/slow-release combination (FS), and double dose fast-release (FF). The corresponding equilibrium ClO₂ concentration in the headspace was about 2, 4, 6 and 8 ppm, respectively. Treatment with F reduced populations of *E. coli* and *A. alternata* by 2.9–4.7, and 1.6 to 4.0 log CFU/g, respectively, within 14 days storage at 20 °C. FS and FF treatments showed little benefit over F. The F and FF treatments reduced population of *S. enterica* for inoculated cherry tomatoes by 3.28 and 3.80 log CFU/g, respectively, compared to control after 14 days' storage at 20 °C. ClO₂-treated grape tomatoes retained higher firmness and had less weight loss compared to the control. The results indicate that 2 ppm of ClO₂ (S) in the clamshells did not adequately control microbial populations; the minimum effective concentration of ClO₂ was 4 ppm (F). Higher concentrations provided a small but incremental improvement in ability to control microorganisms. ClO₂ released into packages of cherry tomatoes during storage reduced weight loss, while maintaining firmness.

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1. Introduction

Fresh produce contaminated with a bacterial agent accounted for 27% of all foodborne illnesses, and was responsible for 35% of all related instances of hospitalizations and 25% of all related deaths in the decade between 1998 and 2008 (Painter et al., 2013). Tomatoes have been associated with several multistate outbreaks of human pathogens and are thus considered to be high risk produce items (Lu & Wu, 2010). Tomatoes are also susceptible to postharvest decay caused by fungal plant pathogens (Wang et al., 2010), which has resulted in serious economic losses for the tomato industry (Lu & Wu, 2010). *Alternaria alternata* is a fungus which causes black mold rot on tomatoes and consequently substantial postharvest

losses (Estiarte, Crespo-Sempere, Marin, Sanchis, & Ramos, 2016). Furthermore, *A. alternata* produces mycotoxins that are harmful to humans and animals (Loghmani, Raoofi, Ownagh, & Dehrezh, 2017). Therefore, effective postharvest decontamination technologies are needed to control foodborne pathogens and prevent postharvest decay in fresh produce.

Chlorine dioxide (ClO₂) is an effective fumigation agent with strong oxidization ability and a broad antimicrobial spectrum (Trinetta, Vaid, Xu, Linton, & Morgan, 2012). ClO₂ gas is ideal for indoor decontamination due to its high penetrability and diffusivity. The antimicrobial efficacy of ClO₂ gas has been evaluated in many previous studies. Approximately a 4.5 log CFU reduction per inoculated strawberry of *Escherichia coli* O157:H7, *L. monocytogenes*, and *Salmonella enterica* was achieved by treatment with 5 mg/L ClO₂ for 10 min (Mahmoud, Bhagat, & Linton, 2007). More than a 5-log reduction of *E. coli* O157:H7 was achieved by 1.24 mg/L ClO₂ gas treatment for 30 min on inoculated green peppers (Han, Sherman,

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Linton, Nielsen, & Nelson, 2000). Additionally, exposing inoculated lettuce leaves to 4.3 mg/L ClO₂ gas for 30 min significantly reduced the population of *E. coli* O157:H7 and *S. Typhimurium* (Lee, Costello, & Kang, 2004).

Published ClO₂ applications on fruits and vegetables have mostly focused on the inactivation of human and plant pathogens combining a relatively high dose (5–10 mg/L) and short exposure time (1–25 min) (Trinetta, Morgan, & Linton, 2010). However, the high dose of ClO₂ caused many adverse effects to produce quality, such as to appearance and taste. Our previous research showed that when packaging fruit with a slow release ClO₂ packet in a clamshell, the fruit quality and safety were remarkably improved (Sun et al., 2014). The concentration of ClO₂ in the clamshell usually was low (less than 8 ppm) and exposure time was as long as the fruit remained in the clamshell, or until the ClO₂ became completely depleted, whichever time period was shorter. However, the exact concentration of ClO₂ in the packaging was not monitored, and the responses of fruit and associated microbial population to the dose × time combination have not been explored. The aim of this study is to investigate the effect of various chlorine dioxide treatments during storage on the survival and persistence of *E. coli*, *A. alternata*, or *S. enterica* inoculated on fresh tomatoes, as well as on the quality and shelf life of tomatoes. The quality was assessed by measuring the firmness and monitoring the weight loss of grape tomatoes during storage.

2. Materials and methods

2.1. Fruit

Fresh grape tomatoes (*Lycopersicon cerasiforme* cv. 'Authentic', average size 12 g each), obtained from a local store, were used for experiment I, and cherry tomatoes (*Lycopersicon cerasiforme* cv. 'Super Sweet 100', average size 8 g each), purchased from a local wholesaler, were used for experiment II. All fruits selected for the experiments were defect-free with similar size and color.

2.2. Pathogens

In experiment I, inoculum was prepared from strains of *Escherichia coli* (wild type, non-pathogenic) and *Alternaria alternata* (avirulent) previously isolated from citrus fruit surfaces (Narciso, Ference, Ritenour, & Widmer, 2012) and stored at –80 °C on *E. coli* agar (ECA) [EC Broth with 1.5% agar] and potato dextrose agar (PDA) plugs in 10% glycerol (as cryoprotectant), respectively. *E. coli* was re-cultured from the frozen ECA plugs on to ECA kept at 35 °C for 24 h, and then re-cultured to a new ECA plate for another 24 h at 35 °C before use. Revived bacterial cultures were confirmed by sampling the ECA plates with a bac-loop, streaking the bacteria on Levine eosin methylene blue (EMB) agar, and incubating for 24 h at 35 °C. Cultures that grew reflective metallic green colonies on EMB indicator agar were confirmed as positive for *E. coli*. *A. alternata* was re-cultured on the PDA plugs at 25 °C for 7 days. The *E. coli* cells and *A. alternata* spores were scraped from the culture media, and suspended in 2 L of sterile distilled water at 20 °C, and 2 mL of Tween-20 was added to improve the suspension. The final *E. coli* population was 7.5 log CFU/mL and *A. alternata* was 5.5 log CFU/mL.

In experiment II, *Salmonella enterica* Newport FDA strain 2757 was grown in tryptic soy broth (TSB) (Becton Dickenson, Sparks, MD) overnight at 35 °C (Zhou et al., 2014). A single colony of the culture was then inoculated into TSB containing appropriate antibiotics and incubated overnight at 35 °C (Zhou et al., 2014). Bacterial cells were harvested by centrifugation, washed once in sterile phosphate buffered saline (PBS; Fisher Bioreagents, FairLawn, NJ), and then suspended in PBS. This cell suspension was diluted to a

final concentration of 7 log CFU/mL.

2.3. Experimental design

2.3.1. Experiment I

Two liters of inoculum, containing either *E. coli* or *A. alternata*, was sprayed using a trigger spray bottle onto 7 kg of grape tomatoes that had been placed in a 10-L stainless steel pan. After 5 min, the excess inoculum was drained, and the fruit were air dried on sterile steel mesh for 2 h before packaging tomatoes (200 g) in 1-pound perforated clamshells (OSU #1, Packaging Plus, Yakima, WA). The ClO₂ pouches were prepared by heat sealing semi-permeable plastic film (selected for chosen release rate) containing 0.5 g Curoxin® ClO₂ slurry (9.5% a.i.). The pouches were then inserted into a porous nonwoven fabric over-pouch, which was then heat sealed. The effective surface area was 6 cm². The pouches were attached to the inside of the lid of each clamshell with double-sided tape and the following five treatments were applied: single dose fast-release (F), single dose slow-release (S), single dose fast/slow combination (FS), double dose fast-release (FF), and non-ClO₂ control (C). Each treatment contained three replicates. The clamshells were stored at 20 °C with 75% relative humidity (RH) for 14 days. The microbial populations, fruit firmness, weight loss, and ClO₂ concentration were recorded on days 0, 3, 7, 10, and 14. The experiment was conducted using three replicates for each treatment.

2.3.2. Experiment II

Cherry tomato fruits were immersed in 1-L inoculum containing approximately 7 log CFU/mL of *S. enterica* inoculum for 5 min. After draining and drying in a bio-safety hood for 30 min, 200 g tomatoes were packed in 1-pound perforated clamshells. Single dose fast-release (F) and double dose fast-release (FF), the best two treatments selected from Experiment I, were applied in this experiment. The inoculated tomatoes in clamshells without ClO₂ pouches served as controls. Tomatoes were stored at 20 °C with 75% RH for 14 days. The microbial populations were assayed on days 0, 4, 7, and 14. The experiment was conducted using three replicates for each treatment.

2.4. Concentration of ClO₂

Chlorine dioxide concentration inside the clamshell was measured by a PortaSens II ClO₂ gas detector (Analytical Technology, Inc., Collegeville, PA).

2.5. Microbiological analysis

For experiment I, 5 fruit samples (about 60 g) from each replicate were transferred under sterile conditions to a sterile sampling bag along with 99 mL of sterile potassium phosphate buffer (0.01 M, pH 7.2) and agitated at 100 rpm for 1 h on an orbital shaker (Innova 2100, New Brunswick Scientific, New Brunswick, NJ). Serial dilutions of the wash were prepared and cultured on different media for microbial counts. ECA media was used for enumerating *E. coli*, and PDA was used for *A. alternata*, using an Eddy Jet Spiral Plater (Neutec Group Inc., Farmingdale, NY). ECA and PDA were incubated at 35 °C for 24 h, and 25 °C for 3 days, respectively (Sun et al., 2014), and the results were read on a ProtoCOL colony counter (Synoptics, Ltd., Cambridge, UK). The populations were expressed as log CFU per gram of fruit. All tests were run in triplicate.

For experiment II, 10 fruit samples (about 80 g) from each replicate (clamshell) were transferred under sterile conditions to a stomacher bag and macerated for 2 min at 230 rpm in 99 mL of 0.01 M sterile phosphate buffer (pH 7.2) using a stomacher blender

(Seward 80 Biomaster, Brinkmann Seward, Ontario, Canada). Serial dilutions (0.1 mL) of fruit buffer wash were spread on 4 XLT4 agar plates, and incubated at 37 °C for 24 h. The colonies were enumerated using the ProtoCol colony counter.

2.6. Firmness and weight loss

Fruit firmness was measured by a FirmTech 2 Fruit Firmness Tester (Bioworks Inc, Wamego, USA) to compress fruit 1 mm, and expressed as $N \cdot m^{-1}$. Twenty grape tomatoes were used per replicate.

To determine weight loss, grape tomatoes in the whole clamshell were weighed on day 0 and on each evaluation day during storage. Weight loss was expressed as the percentage loss of the initial total weight.

2.7. Statistical analyses

All experiments were replicated in triplicate. Data were analyzed according to a two factor linear model with storage time and treatment as the two factors. Analysis of variance (ANOVA) was performed using SPSS version 17.0 software (Experian QAS, Boston, MA). Different samples were analyzed on each evaluation day for all analyses. Assumptions of normality and variance homogeneity were checked. Microbial data were log transformed prior to analysis. Mean separation was determined by Duncan's multiple range test. Significance was defined at $p < 0.05$.

3. Results and discussion

3.1. Concentration of ClO_2

The release property of ClO_2 pouches at 20 °C in 1-pound perforated clamshells containing 200 g grape tomatoes is shown in Fig. 1. Generally, ClO_2 released quickly and reached a high concentration by day 3, and the concentration remained relatively stable for the next 7 days for all the treatments except FS. For FS treatment, the ClO_2 concentration declined from day 3 to day 7, was

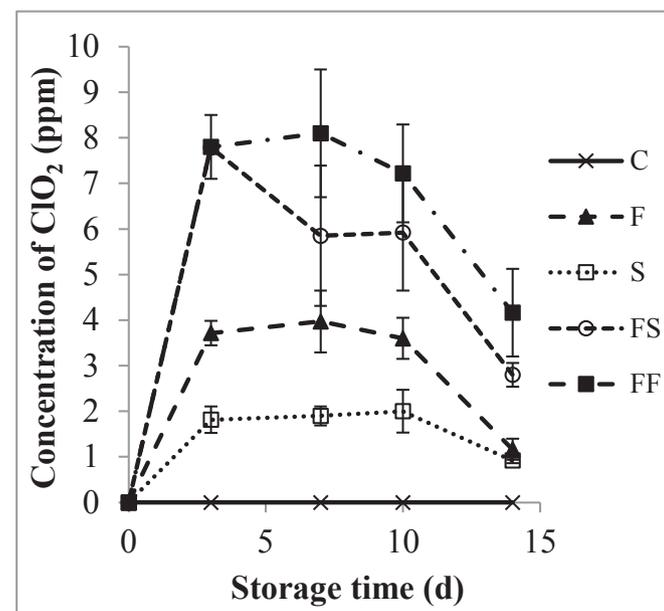


Fig. 1. Concentration of ClO_2 in 1-pound perforated clamshell packaging with 200 g grape tomatoes at 20 °C. (C: control; F: single dose fast-release; S: single dose slow-release; FS: single dose fast/slow combination; FF: double dose fast-release.)

stable from day 7 to day 10, and began to decline again after day 10. The FF treatment showed the highest maximum ClO_2 concentration of all treatments (about 8 ppm on d 7), followed by FS (about 6 ppm), F (about 4 ppm), and then S (about 2 ppm). The concentration of all treatments declined dramatically between day 10 and day 14.

The concentration of ClO_2 in a clamshell is determined by differences in rates of gas release from the ClO_2 pouch and gas losses due to degradation and mass flow out of the perforated fruit package. Following a rapid increase during the early stage, ClO_2 concentration reached equilibrium, then decreased after the ClO_2 from the source pouch was depleted (Fig. 1). Many factors, such as temperature, RH, light, and the presence of fruit can affect ClO_2 release and loss rates: the diffusion rate of ClO_2 increases with increasing temperature (Lee, Burgess, Rubino, & Auras, 2015), ClO_2 degrades at a higher rate due to higher RH (Jeon, Lee, Lee, & Yu, 2012; Lee et al., 2015; Park & Kang, 2015a), ClO_2 degrades more quickly under more intense light (Lee et al., 2015), and surface moisture on produce increases the degradation rate of ClO_2 (Smith, Ernst, & Herges, 2015). In this research, we successfully manipulated the ClO_2 level by a simple regulation of the pouch parameters (mass of Curoxin ClO_2 slurry, film permeability, and pouch quantity) (Fig. 1).

3.2. Antimicrobial activity of ClO_2

In experiment I, the single dose fast-release (F) treatment reduced populations of *E. coli* and *A. alternata* by 2.9–4.7, and 1.6 to 4.0 log CFU/g, respectively compared to control, within 14 days storage at 20 °C (Fig. 2). The single dose slow-release (S) showed the least antimicrobial activity, possibly due to the lowest concentration of ClO_2 in clamshell packaging (Fig. 1). The single dose fast/slow combination (FS) and double dose fast-release (FF) treatments showed strong antimicrobial activity against *E. coli* as did F. Both F and FF treatments completely inhibited the growth of *A. alternata* after 10 days storage. Additionally, F treatments showed better antimicrobial property than FS after 14 days storage. Therefore, the F and FF treatments were selected for the experiment II. The F and FF treatments reduced populations of *S. enterica* by 3.28 and 3.80 log CFU/g, respectively compared to control within 14 days storage at 20 °C (Fig. 3).

Chlorine dioxide has emerged as a sanitizing treatment for fruits and vegetables in recent years (Bhagat, Mahmoud, & Linton, 2010). Gaseous ClO_2 shows higher penetrability than in its aqueous form (Gomez-Lopez, Rajkovic, Ragaert, Smigic, & Devlieghere, 2009). An over 5-log reduction in CFU per potato of natural microbiota was achieved with a gaseous ClO_2 treatment at a concentration of 40 mg/L after 5 h (Wu & Rioux, 2010). High-concentration-short-time gas treatments have been applied for the inactivation of *S. enterica* spp. on tomatoes, and a higher than 4-log CFU/g reduction of *S. enterica* spp. was achieved after 10 mg/L ClO_2 gas treatments for 3 min at 25 °C with 90–95% RH on Roma tomatoes (Trinetta et al., 2010). Treatment with ClO_2 gas at 0.4 mg/L for 4 h at 13 °C with 90% RH reduced *S. enterica* Montevideo and *S. enterica* Typhimurium populations by 4.6 and 5 log CFU/g, respectively (Olanya, Annous, & Taylor, 2015). Treatment with ClO_2 gas at 10 ppm for 20 min at 22 °C with 90% RH resulted in 3.9, and 3.5 log CFU/g reductions of *E. coli* O157:H7 and *S. enterica* Typhimurium on tomatoes (Park & Kang, 2015b). Mycelial growth of *A. alternata* was completely inhibited by 10 mg/L of ClO_2 gas treatment for 3 min at 23 °C *in vitro*, and the decay of Roma tomatoes caused by *A. alternata* was significantly ($p < 0.05$) delayed after the same ClO_2 gas treatments for 7 min (Trinetta, Linton, & Morgan, 2013). Our results confirmed the antimicrobial activity of ClO_2 gas against *E. coli*, *S. enterica*, and *A. alternata*. In our experiment, the higher

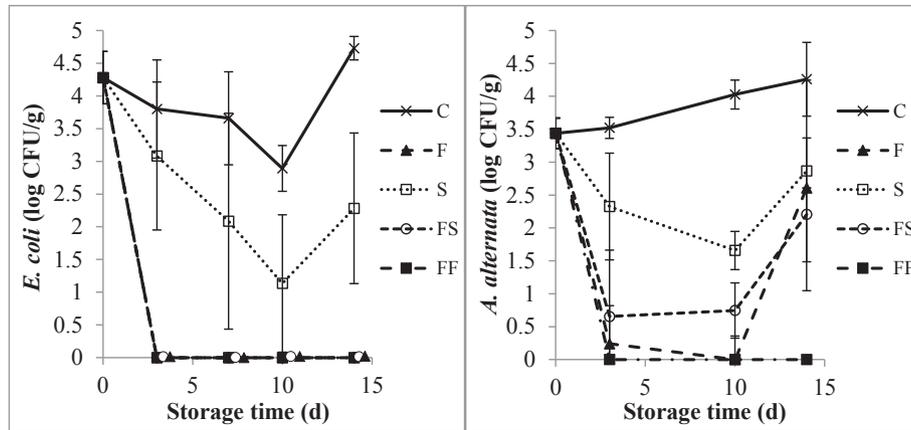


Fig. 2. Effects of ClO_2 on *E. coli* (A) and *A. alternata* (B) population of cherry tomatoes at 20 °C for up to 14 days, expressed in log CFU/g. (C: control; F: single dose fast-release; S: single dose slow-release; FS: single dose fast/slow combination; FF: double dose fast-release. Left panel: lines for F, FS and FF overlapped.)

antimicrobial activity of F and FF treatments against *E. coli*, *S. enterica*, and *A. alternata* was associated with the higher ClO_2 concentration in the clamshell.

3.3. Firmness and weight loss

Grape tomatoes treated with ClO_2 were firmer than the control fruit (Fig. 4). All the treatments (except FS) reduced the weight loss of grape tomatoes significantly (Fig. 5). The F and FF treatments reduced weight loss by about 2.5%, and the S treatment reduced weight loss by more than 3%. Water loss reduces turgor pressure, which can result in loss of firmness (Saladie et al., 2007). A linear relationship between softening and weight loss was demonstrated in blueberries (Paniagua, East, Hindmarsh, & Heyes, 2013). ClO_2 has been suggested to reduce fruit metabolism as well as prevent weight loss and retain firmness (Gomez-Lopez, Ragaert, Jeyachandran, Debevere, & Devlieghere, 2008). Similar to our results, ClO_2 gas treatments significantly slowed weight loss of strawberries (Wang et al., 2014).

Firmness is an important quality factor for preparation and sale of fruit (Leiva-Valenzuela, Lu, & Aguilera, 2013). Maintenance of firmness by ClO_2 treatment has been observed for other fruits, such as blueberries (Sun et al., 2014), and strawberries (Wang et al., 2014). We found that ClO_2 delayed tomato softening, which did not contradict the finding of Liu et al. (Liu et al., 1993) that decay caused by *A. alternata* resulted in softening of tomatoes. In addition to affecting firmness by maintenance of turgor pressure (by reducing water loss) and inhibiting microbial growth, ClO_2 treatment has been shown to inhibit fruit enzyme activities such as peroxidase and polyphenol oxidase, which have been determined to have important roles in the softening process that accompanies senescence (Wang, Wu, Ma, & Ding, 2011). ClO_2 has also been shown to inhibit production of the ripening hormone, ethylene, by reducing the expression of ethylene biosynthesis related genes (*LeACS2*, *LeACS4* and *LeACO1*) (Guo et al., 2014), for which the resulting delay of ripening also results in delay of softening as a part of the ripening process. More specifically, the changes in cell wall

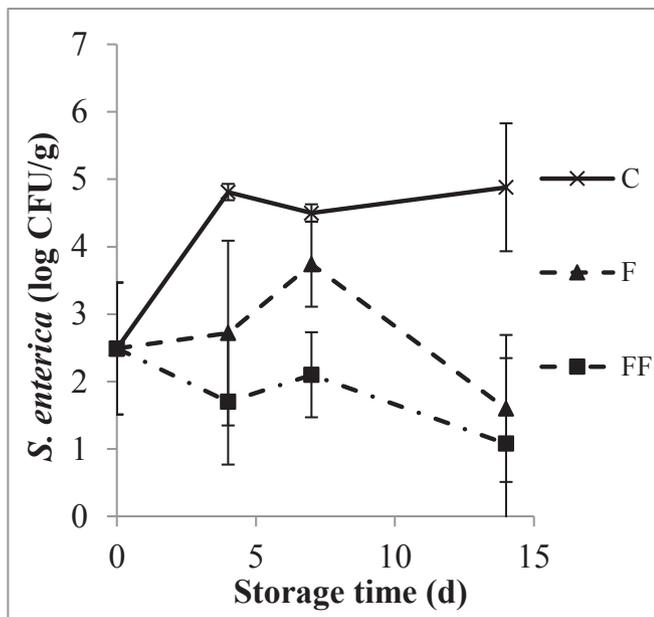


Fig. 3. Effects of single/double dose fast-release ClO_2 pouches on persistence of *S. enterica* on grape tomatoes at 20 °C for up to 14 days, expressed in log CFU/g. (C: control; F: single dose fast-release; FF: double dose fast-release.)

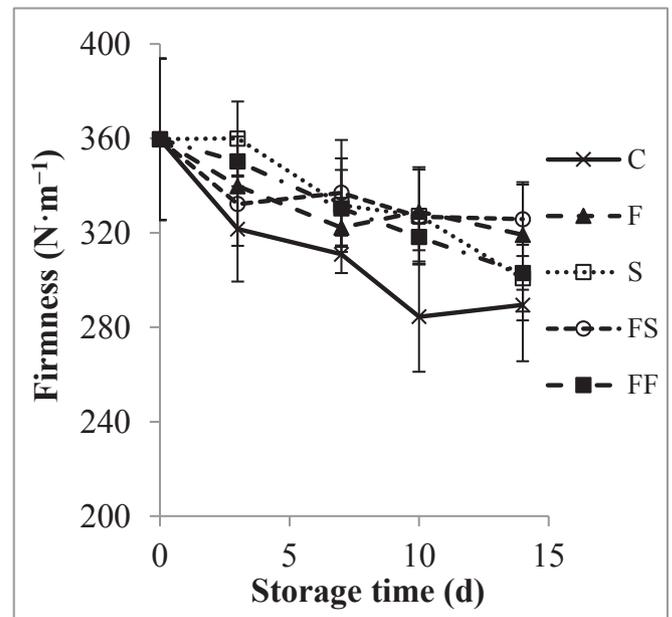


Fig. 4. Effects of ClO_2 on firmness of cherry tomatoes at 20 °C for up to 14 days, expressed in $\text{N}\cdot\text{m}^{-1}$. (C: control; F: single dose fast-release; S: single dose slow-release; FS: single dose fast/slow release; FF: double dose fast-release.)

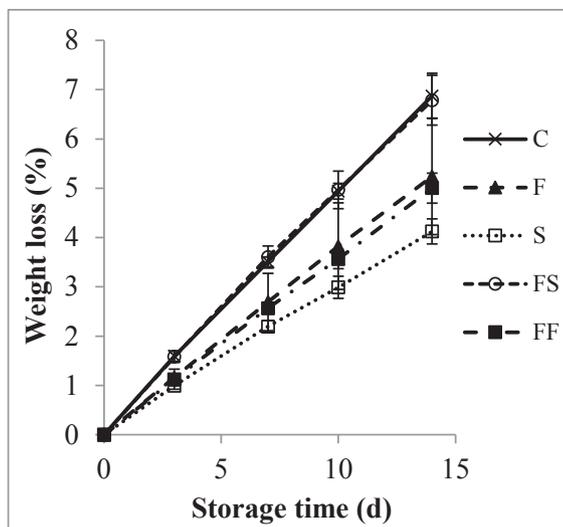


Fig. 5. Effects of ClO₂ on weight loss of cherry tomatoes at 20 °C for up to 14 days, expressed in %. (C: control; F: single dose fast-release; S: single dose slow-release; FS: single dose fast/slow combination; FF: double dose fast-release.)

polysaccharides during fruit ripening alter the chemical structure of pectin and reduced fruit firmness (Bonnin & Lahaye, 2013). Since ClO₂ inhibited cell wall protein synthesis, it could possibly decrease the cell wall changes involved in fruit softening (Mahmoud, Vaidya, Corvalan, & Linton, 2008).

4. Conclusion

Our results indicate that ClO₂ possesses strong antibacterial and antifungal activities on inoculated cherry/grape tomatoes. The findings suggest that application of ClO₂ at low concentrations for long durations in active packaging is useful to improve fruit safety, reduce decay and weight loss, and maintain firmness during storage.

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