Plant Physiological Response of Strawberry Fruit to Chlorine Dioxide Gas Treatment during Postharvest Storage

ZHE WANG, JAN NARCISO*, ALICE BIOTTEAU, ANNE PLOTTO, AND JINHE BAI

USDA, ARS, USHRL, 2001 S. Rock Road, Ft. Pierce, FL 34945

Additional index words. ClO₂, timed-release, weight loss, fruit firmness

Chlorine dioxide (ClO₂), a strong oxidizing and sanitizing agent, is used as a postharvest sanitizer for fruits and vegetables and is generally applied on a packing line using a ClO₂ generator. The objective of this research was to study the physiological responses of strawberries to ClO₂ when applied to the fruit using a crystalline form packaged in a “sachet” attached to a polyethylene clamshell. The ClO₂ gas was released gradually during storage and distribution. Strawberries were packed in commercial clamshells, with or without ClO₂ treatments, and stored at 1 and 5 °C for 14 d to simulate cold storage/shipping conditions and at 10 and 20 °C for 7 d to simulate refrigerated and non-refrigerated shelf conditions. The effect of ClO₂ on strawberries was assessed by determining weight loss, firmness, surface color, soluble solids content (SSC), titratable acidity (TA), respiration rate, and ethylene production. Chlorine dioxide concentration at 0.5 g per clamshell markedly slowed weight loss and softening of strawberry fruit at 10 °C or below. This effect disappeared when ClO₂ dosage was 0.2 g per clamshell at any temperature, or storage temperature was 20 °C at any ClO₂ dosage. Soluble solids content and TA were stable during storage regardless of storage temperature and ClO₂ treatment. Surface color values were not significantly different between treatments.

Strawberries are a popular fruit rich with nutrients (amino acids, vitamins and anthocyanins), high visible appeal and desirable flavor, but are highly perishable and have relatively high physiological activities (Campaniello et al., 2008). Such behaviors result in a rapid deterioration in quality such as fungal decay, softening and shrinkage, discoloration, off-flavors, and short storage life (Campaniello et al., 2008; Perdones et al., 2012; Romanazzi et al., 2013; Steen et al., 2002; Vachon et al., 2003; Yang et al., 2010). Water loss, which can reach 40% during storage, plays a major rule in quality deterioration of strawberries. At present, the most prevalent method of maintaining quality and controlling decay is rapid cooling after harvest and storage at low temperatures (Han et al., 2004; Mall and Grossmann, 2003). Any handling of the berries postharvest for sanitation or preservation purposes, results in damage and shortens shelf life (Romanazzi et al., 2013).

Chlorine dioxide has received great attention as a decontaminant for produce, largely because its efficacy is less affected by pH and organic matter than liquid chlorine and hypochlorites and does not react with ammonia to form chloramines. (Artés et al., 2009; Gómez-López et al., 2007, 2009; Simons and Sanguansri, 1997; Trinetta et al., 2010). Chlorine dioxide is a strong oxidizing and sanitizing agent, which has broad and efficient biocide effectiveness at low concentrations (Singh et al., 2002; Tomás-Callejas et al., 2012). Chlorine dioxide is classified as a noncarcinogenic product (Richardson et al., 2007; Tomás-Callejas et al., 2012), and the use of ClO₂ has risen as an antimicrobial treatment for fruits and vegetables (Burton et al., 2013; Du et al., 2003). The U.S. Environmental Protection Agency (EPA) and U.S. Food and Drug Administration (FDA) have allowed ClO₂ for use as a disinfectant, sanitizer, and sterilant for fruits and vegetables.

In recent years, the vast majority of published information describes the effect of ClO₂ on the reduction of foodborne infection. Mahovic et al. (2007) and Trinetta et al. (2010) have shown that ClO₂ gas can reduce risk of microbial contamination while maintaining fresh fruit attributes, and was effective for controlling postharvest decay of tomatoes. Du et al. (2007) showed that ClO₂ gas treatment could effectively delay the postharvest physiological deterioration of green peppers, inhibited decay and respiration, and maintained nutritional and sensory quality. It was observed by Lee et al. (2004), Singh et al. (2002), Du et al. (2003), Han et al. (2004), and Mahmoud et al. (2007) that ClO₂ gas treatment significantly reduced E. coli O157:H7, which has emerged as a foodborne pathogen of major public health concern. Mahmoud et al. (2007) found that the inactivation mechanism of ClO₂ was to disrupt protein synthesis and increase the permeability of the outer pathogen membrane by reacting with the membrane protein and lipids.

The objective of this research was to study the physiological response of strawberries to ClO₂ when it was applied to the fruit in a crystalline form in a sachet placed in the clamshell. The effect of ClO₂ on strawberries was assessed by determining weight loss, firmness, surface color, soluble solids content (SSC), titratable acidity (TA), respiration rate and ethylene production.

Materials and Methods

Four experiments were conducted over 3 years (2011–13). Two-variable (ClO₂ vs. control) comparison designs were used in all experiments. Fruit were packaged in 1-lb clamshells with or without a ClO₂ pad. The ClO₂ pad was made with a specified amount of a crystalline form of ClO₂ (Worrer Water Technologies, Charlottesville, VA) placed in a permeable envelope that allowed ClO₂ vapor to diffuse gradually. The pads were attached on the top lids of the clamshells with a double-sided sticky tape. Fruit

*Corresponding author; phone: (772) 462-5848; email: jan.narciso@ars.usda.gov
in the clamshells without ClO₂ pads were stored in a separate storage room from fruit with pads to avoid contamination with ClO₂. Storage temperatures were 1, 6, 10, and 20 °C to simulate ideal storage and shipping conditions, and commercial storage and display in groceries respectively. Each treatment included three replicates. Water loss, firmness, color, and other quality and physiological parameters were measured during storage at the different temperatures.

**Plant and packaging materials.** Strawberry fruit cv. Festival was used for all four experiments. For experiment I, fruit were harvested at commercial maturity from a commercial farm located in Haines City, FL. For experiment II, III, and IV, strawberries were ordered from a local supermarket. All fruits, free from defects and uniform for ripening stage (full red color) and fruit size, were selected and pooled for each experiment.

The commercial 1-lb polyethylene terephthalate clamshells (PET #1, Packaging Plus, Yakima, WA) were used for fruit containment. The clamshells had openings on bottom and side walls.

**Experimental conditions**

**Experiment I.** In Mar. 2011, fruit (10 kg) were randomly packaged in 21 clamshells. Each clamshell contained about 450 g of fruit. Three clamshells were used for initial (day 0) measurements. The rest of the clamshells were divided to two groups: ClO₂ treatment (0.5 g per clamshell) and control. Fruit in clamshells, with or without ClO₂ pads, were stored at 1, 10, and 20 °C for 10, 6 and 3 d, respectively. Each treatment contained three clamshells as three replicates. Measurements were taken at end of the storage.

**Experiment II.** In Jan. 2012, fruit (14 kg) were randomly packaged in 39 clamshells (≈350 g per clamshell). Dosage of ClO₂ was 0.5 g per clamshell. Three clamshells were used for initial (day 0) measurements. The rest of the clamshells were divided into the following treatments/replicates: two treatments (ClO₂ vs. control) × two storage temperatures (6 °C and 20 °C) × three sampling times (day 6, 8, and 13 for 6 °C and day 1, 2, and 3 for 20 °C) × three replicates.

**Experiment III.** In Feb. 2012, fruit (7.5 kg) were randomly packaged in 21 clamshells (≈350 g per clamshell). Dosage of ClO₂ was 0.5 g per clamshell, and storage temperature was 6 °C. Clamshells were divided into the following treatments/replicates: two treatments (ClO₂ vs. control) × three sampling times (day 4, 8, and 10) × three replicates + three initial samples (for day 0).

**Experiment IV.** The experiment was conducted in Mar. 2013, and treatment settings were similar to Experiment II except that the ClO₂ dosage was changed to 0.2 g instead of 0.5 g per clamshell.

**Quality parameters**

**Weight loss.** To determine weight loss, fruit in the same clamshells were weighed at the beginning and during storage at different temperatures. Weight loss was expressed as the percent loss of the initial total weight.

**Fruit surface color.** Ten fruits were taken from each replicate for the measurement of surface color using a colorimeter (model CR-300, Minolta, Tokyo, Japan). The instrument was calibrated using a white tile. Color was expressed as CIE L*, a*, b*, C*, and hue angle value.

**Firmness.** Fruit firmness was assessed using a FirmTech 2 Fruit Firmness Tester (Bioworks Inc, Wamego, KS), and expressed as Newton (N) m⁻¹ (force used for pressing fruit by 1 m). Ten fruit were used per replicate.

**Soluble solid content (SSC) and titratable acidity (TA).** Ten fruit per replicate were homogenized, and centrifuged. The supernatant was used for SSC and TA analysis. Soluble solids content was measured using a refractometer (RX-5000, Atago Co. Ltd., Tokyo, Japan), and expressed as °Brix and TA was determined by titrating a mixture of 10 mL juice and 40 mL ion-free water using a Metrohm system (808 Titrando, Metrohm Inc., Tampa, FL), and expressed as percent citric acid.

**Respiration rate and ethylene production.** Strawberry samples (≈300 g) were placed in a 1-L glass jars, hermetically sealed with a rubber stopper for 30 min. Headspace gas samples were obtained of the jar headspace using a 10-mL gas-tight syringe and injected into a gas chromatograph (GC, Model 5890A, Hewlett-Packard, Wilmington, DE) for CO₂, O₂, and ethylene measurements. For CO₂ and O₂ measurements, a thermal conductivity detector was used. The column was model CTR 1 (Alltech Associates, Inc., Deerfield, IL), which consists of a 3-mm-diameter inner polymer column for CO₂ and a concentric 4-mm molecular sieve outer column. This column gives separate peaks for CO₂ and O₂. The CO₂ and O₂ concentrations of samples were determined by peak areas, compared with areas of known standards. Column temperature and flow velocity were 40 °C and 3.0 cm·s⁻¹, respectively. For ethylene analysis, GC was equipped with an activated alumina column and a flame ionization detector and peak areas were compared with known ethylene standards.

**Statistical analyses.** Each value is the average of three replicates. For attributes of color values and firmness, each replicate was the average of 10 strawberries. Differences between samples were analyzed with a two independent sample t-tests, using Excel and Origin software (OriginLab Corporation, Northampton, MA) and were considered to be significant when P < 0.05.

**Results and Discussion**

**Weight loss.** All fruits exhibited a continuous weight loss over the storage period regardless of storage temperature or ClO₂ treatment (Fig 1). Generally, the higher the storage temperature, the higher the weight loss rate.

In the 2011–12 experiments, the ClO₂ treatment dosage was 0.5 g per clamshell (Fig. 1) which significantly (P < 0.05) decreased weight loss of fruit compared to the control at 1, 6, and 10 °C. In 2013, ClO₂ dosage was reduced to 0.2 g to test if a lower ClO₂ concentration could decrease weight loss of fruit as well as the higher concentration. Unfortunately, this reduced concentration did not protect fruit from water loss or decay and did not affect firmness. Thus, the dose of ClO₂ is an important factor for decreasing weight loss of fruit.

Weight loss of fruit is mainly due to the water vapor transpiration process between the fruit and surrounding air (Valero et al., 2013). This process includes the transport of moisture from fruit intercellular spaces through the thin epidermis of the fruit resulting in the evaporation of moisture from the fruit surface (Paniagua et al., 2013).

**Firmness.** During fruit ripening one of the most notable changes is softening, which is related to biochemical alterations at the cell wall, middle lamella and membrane levels. Pectic enzymes, polygalacturonase and pectin methylesterase have been attributed to a significant role in the softening process (Paull et al., 1999). Effect of ClO₂ treatment on fruit firmness is presented in Fig. 2. Similar to weight loss, ClO₂ at 0.5 g significantly slowed loss of firmness, but not at 0.2 g. At 0.5 g, ClO₂ worked well at 10 °C or lower (Fig. 2). Aghdama et al. (2012) and Paniagua et al. (2013) have concluded that the changes in firmness can be...
attributed to water loss, significant shrinkage and fungal infection, which led to more pronounced tissue senescence and cell wall breakdown. Many studies (Alejandro et al., 2012; Du et al., 2003; Gómez-López et al., 2008, 2009) have shown that ClO₂ could slow down fruit metabolism, in addition to inhibiting weight loss, and Mahmoud et al. (2008) showed that ClO₂ significantly reduced E. coli (H7:O157). Some research indicates that ripening alters overall wall strength and reduces fruit firmness (Bonnin and Lahaye, 2013; Rose et al., 1998). Mahmoud et al. (2008) showed that ClO₂ inhibited cell wall protein synthesis, thereby reducing fruit softening.

**COLOR.** The changes of L*, C* and Hue angle values for strawberries, from high to low, represent lightness to darkness, vividness to dullness, and red to yellow color, respectively (Romanazzi et al., 2013; Shin et al., 2012). In the present investigation, there was no significant difference among treatments (data not shown).

**Soluble solids content (SSC) and titratable acidity (TA).** There were no obvious changes of SSC and TA between the control and samples with ClO₂ treatment during the storage in experiment (data not shown).

**Respiration rate and ethylene production.** The respiration rate of samples was similar in ClO₂ treated fruit and the control. Ethylene production rate of all the samples was undetectable over the storage period at 6 °C, and there were no significant differences between control and treated samples at 20 °C (data not shown). It was concluded that ClO₂ did not stimulate ethylene production or...
respiration. Ma et al. (2012) and King and Morris (1994) showed that ethylene production boosts senescence in fruit. If ethylene production is not stimulated by ClO₂, then senescence and accompanying fruit degradation is slowed allowing an explanation for firmness retention in the strawberries treated with ClO₂.

**Conclusion**

In addition to sanitation, ClO₂ treatment at 0.5 g markedly slowed weight loss, and softening of strawberries most likely due to delayed senescence. Together with the sanitation effect, ClO₂ is a very powerful tool for postharvest handling and storage in strawberry. As strawberry is a highly perishable horticultural fruit with a short shelf life at room temperature, the extension of shelf life and maintenance of visual quality postharvest with ClO₂ treatments will contribute to reducing postharvest economic loss for the industry and provide fresher produce for consumers.

**Literature Cited**


