Postharvest Responses of Fresh Peaches to Gaseous Ozone Treatments During Cold Storage

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Abstract

Ozone is a self-decomposing gas with disinfecting capabilities that presents challenges in maintaining and controlling specific concentrations. It is typically generated on-site through corona discharge technology. This study investigated the effect of low gaseous ozone (O_3) concentrations (0.5 and 1.0 ppm) on the postharvest quality and shelf life of peaches during cold storage. Two commercial peach cultivars, 'O'Henry' and 'August Lady', were stored under controlled conditions of 1.1°C (34°F) with 90-95% relative humidity and exposed to continuous gaseous O_3 treatments throughout the 28 d cold storage period using a timer-controlled delivery system, with untreated fruit serving as the control (CS). Quality parameters, including firmness, total soluble solids (TSS), titratable acidity (TA), TSS/TA ratio, respiration rate (RR), decay incidence, weight loss, visual appearance, and skin color, were assessed after 7, 14, 21, and 28 days of storage. Results indicated that exposure to 1 ppm concentration of gaseous O_3 was not effective in extending shelf life or preserving fruit quality. Instead, 1 ppm O_3 treatment resulted in skin pitting after 14 d of storage for both cultivars. Although fruit treated with 1 ppm O_3 exhibited lower weight loss than the control after 28 d, this benefit did not offset the negative effects on external quality. Both 0.5 and 1 ppm O_3 did not significantly affect decay incidence compared with the control throughout the storage period and maintained decay levels between 0 and 7.1% through the 28 d of cold storage. Also, the lower exposure level of 0.5 ppm lacked pitting incidence symptoms and overall preserved a visual appearance comparable to CS. These findings highlight the limited tolerance of the evaluated peach cultivars to gaseous ozone concentrations above 0.5 ppm, under the tested storage conditions, which increased susceptibility to storage-induced disorders.

Keywords: Quality, Shelf life, Decay, Fruit, Physiology.

1. Introduction

Postharvest studies have focused on enhancing the organoleptic quality and extending the storage potential of fresh fruits and vegetables. Once fresh produce is harvested or separated from the mother plant, quality begins to decline due to physiological and biochemical processes (Sirangelo et al. 2022). Peaches are climacteric fruit, characterized by increased respiration and ethylene production, both of which speed up the ripening process (Kou and Wu 2018; Ai et al. 2024). Limited storage life, quality degradation, and high susceptibility to postharvest diseases remain major constraints in the global peach value chain, directly affecting the fruit marketability and causing significant economic losses (Wang et al. 2017; Yang et al. 2019). Therefore, effective management after harvest is crucial in determining the fate of produce in the market (Mahajan et al. 2014).

Postharvest microbial control is one of the most crucial practices for mitigating foodborne diseases and maintaining the quality of fresh produce during postharvest handling (Issa-Zacharia et al. 2010). Similar to many other fruit and vegetables, peaches have the potential to harbor harmful pathogens and microbes, including *Salmonella, E. coli*, *Staphylococcus aureus,* and *L. monocytogenes,* which can be extremely dangerous to humans if consumed (Duvenage and Korsten, 2017). Besides human pathogens and viruses, peaches can become infected pre- and post-harvest by phytopathogenic diseases, which fall into two main categories based on the mode of infection. The first category is “quiescent,” characterized by a progressively evolving visible infection. The second category is “latent,” which is a non-symptomatic infection where the pathogen infects before harvest in the field (Prusky et al. 2013; Wenneker and Thomma 2020). Postharvest fruit sanitation plays a critical role in minimizing the potential for microbial contamination and the incidence of fresh fruit spoilage caused by fungal diseases. Over the years, the control of these diseases has been managed through the use of fungicides and pesticides. However, their current applications in the postharvest fruit storage are extremely limited due to toxicity issues (Pétriacq et al. 2018).

Recently, there has been increasing attention on alternative strategies to fungicides that can reduce disease incidence while extending shelf life and, most importantly, preserving the fresh quality of the fruit. Ozone (O_3) is a byproduct of oxygen (O_2) molecule dissociation, generating free radicals that react with O_2 molecules to form O_3 (Heim and Glas, 2011). It has gained interest as a rapidly decomposing gas that quickly breaks down into atmospheric O_2, leaving no residues (Batakliev et al. 2014; Epelle et al. 2023), and it has long been used as a disinfectant agent for packing lines, process water, and surface disinfection of fresh produce or storage rooms (Fan, 2021; Suslow, 2004). The United States Department of Agriculture (USDA) has listed O_3 on the national list of allowed substances as an ingredient in or on processed products labeled as "organic" (Hamil 2017). On the other hand, the US Food and Drug Administration (FDA) recognizes it as an antimicrobial additive for direct food contact in both gas and aqueous forms (Sarron et al. 2021).

According to Horvitz and Cantalejo (2014), O_3 is a powerful oxidant that effectively inactivates various microorganisms, including *Botrytis* and *Rhizopus* species, on berries. On the other hand, studies have shown that the effects of O_3 on postharvest fruit quality can be either beneficial or detrimental, depending on the dosage applied (Aslam et al. 2020; Chamnan et al. 2022; Tomar and Pradhan 2024; Giannopoulos et al. 2025a). Although numerous studies have highlighted the potential of O_3 applications in postharvest fruit and vegetable processing, the limited research evaluating the effects of low-dose gaseous O_3 applications on different peach cultivars, particularly in relation to quality attributes and decay incidence, underlines the need for further investigation in this area.

Therefore, the objective of this study was to evaluate the effects of postharvest continuous gaseous O_3 applications at 0.5 and 1 ppm on fruit quality and decay incidence in 'O'Henry' and 'August Lady' peaches during cold storage.

2. Materials and Methods

2.1. Peach fruit

Two popular commercial peach cultivars, 'O'Henry' and 'August Lady', conventionally grown in commercial orchards in central Georgia (Fort Valley, GA), were evaluated. Fruit were sourced from commercial packinghouses at commercial maturity, as determined by ground color and firmness typical of melting-flesh freestone cultivars. Peaches underwent standard hydrocooling and sanitation procedures and were then transferred on the same day to the Vidalia Onion Research Laboratory in Tifton, GA. Upon arrival samples were inspected, and any fruit that showed signs of deformity, disease, or damage was discarded.

2.2. Ozone treatments and cold storage

Freshly harvested peaches of 'O'Henry' and 'August Lady' cultivars were stored at 1.1 °C and 90–95% RH for 0, 7, 14, 21, and 28 days under three conditions: exposure to 0.5 ppm gaseous ozone (O_3), exposure to 1 ppm gaseous O_3, and cold storage without O_3 exposure (CS), which represents the standard industry storage practice. The storage temperature was designed to avoid the "killing zone" (2.2-7.7 °C), while using a near-freezing temperature (1.1 °C) commonly applied in commercial cold storage to slow metabolic activity, despite the known risk of chilling injury development during prolonged storage (Crisosto et al. 2008). A commercial O_3 generator (Alpine Air, Provo, UT) was used in combination with the fans of the cooling unit to evenly distribute the O_3 gas, with digital timers regulating its application (Figure 1). Ozone concentrations inside the storage chambers were continuously monitored with a factory-calibrated O_3 gas analyzer (FD-600, Forensics Detectors, Palos Verdes Peninsula, CA, USA). Levels were recorded hourly throughout the storage period to verify O_3 dissipation and ensure stable delivery of the target concentrations (0.5 and 1.0 ppm; ± 0.1 ppm.). Readings were taken at the chamber air circulation outlet, assuming homogeneous O_3 distribution under forced air circulation. To simulate retail or home conditions, fruit were transferred to ambient conditions (20 °C, 70% RH) for two additional days at each evaluation point, allowing chilling injury and other quality defects to develop.

2.3. Postharvest quality evaluation

Postharvest evaluations were carried out at the Vidalia Onion Research Laboratory (121 South Entomology Drive, Tifton, GA), and fruit quality was assessed through destructive and non-destructive techniques. Initial evaluations were performed on day 0. The experimental unit was a group of 30 fruit per treatment subjected to the same O_3 level and storage conditions. Quality attributes were evaluated using fruit sampled destructively, such that different fruit were used at each evaluation point. Unless otherwise stated, ten fruit per replication, per treatment were used for each evaluation at each storage time.

2.3.1. Non-destructive Measurements

Visual Appearance

To assess the visual appearance of the peaches, samples from each treatment were randomly selected, with each fruit acting as an individual replicate. High-quality photographs were captured on days 0, 7, 14, 21, and 28 after storage using a digital camera (Canon DS126741 EOS Rebel, Tokyo, Japan), with fruit placed within an enclosed photobooth to ensure consistent lighting conditions for all images.

Decay Incidence

Decay was defined as the presence of visible fungal growth on the fruit surface., whereas fruit exhibiting ambiguous or superficial symptoms without visible mycelial development were not considered affected. Results were expressed as a percentage of decay incidence.

Respiration Rate

The respiration rate was assessed using a CO_2/O_2 modified atmosphere package gas analyzer (Bridge 900141; Bedford Heights, OH, USA). For each treatment and storage time, 10 fruit per replication were randomly selected and placed into airtight two-gallon plastic containers (United Solutions, Leominster, MA, USA) for 60 minutes incubation period. Fruit mass was recorded for each container prior to measurement. Headspace (free) volume was calculated as the total container volume minus fruit volume, assuming a fruit density of 1 g mL^{-1}. The gas analyzer was zeroed before use, and the

CO_2 concentration was measured at the end of the incubation period. The CO_2 concentration was determined using the following formula (Fonseca et al. 2002).

$$R_{CO_2} = \frac{\left(y_{co_2}^{t_f} - y_{co_2}^{t_i}\right) \cdot V}{100 \cdot M \cdot \left(t_f - t_i\right)}$$

where:

R= respiration (consumption/production) rate, $mL \cdot kg^{-1} \cdot h^{-1}$

V= free volume, mL

y= volumetric concentration, % v/v

M= mass, kg

t= time, h

Skin Color

The skin color was evaluated by selecting 10 random peaches from each treatment. Measurements were obtained with a digital colorimeter (Konica Minolta CR-400, Tokyo, Japan), calibrated prior to use with a standard white reflector plate. Measurements were performed in contact mode using a 12.5-mm aperture under illuminant C with a 10° standard observer. Three readings were taken at random locations on the fruit surface, and their average was considered a single value per fruit. Color values were recorded following the CIE L* a* b* color system, where L* represents lightness (ranging from 0 for black to 100 for white), a* indicates the red-green axis where +a represents a red and -a green spectrum of the palette. The b* denotes the yellow-blue axis where +b represents the yellow and -b the blue spectrum of the palette The C* value represents the intensity and saturation of the color and the h° value is the hue angle of the color from a range of (0° to 360°) (Long et al. 2021).

Weight Loss

All peaches were tagged and weighed on the first day of the experiment. Weight loss (%) was calculated using the difference between the initial and final weights upon each measurement day. Then divided by the initial weight and multiplied by 100 to express the weight loss as a percentage according to the following equation:

$$Weight\ Loss\ (\%) = \frac{(W_i - W_f)}{W_i * 100}$$

Where, Wi is the initial weight (Day 0), and Wf is the final weight.

2.3.2. Destructive Measurements

Firmness

Firmness evaluations were performed following a protocol by Contador et al. (2016) with slight modifications. Measurements were performed using a digital force gauge (AFG 250N Mecmesin, PPT Group UK, West Sussex, UK) attached to a texture analyzer tower (Multitest 2.5-d Mecmesin, PPT Group UK, West Sussex, UK). After removing a thin slide of peel (approximately 1 - 2 mm thick) at the measurement site, fruit were penetrated with an 8 mm cylindrical probe at a loading speed of 5 $mm \cdot s^{-1}$, with a maximum puncture depth of 10 mm. Measurements were conducted after fruit equilibrated to room temperature. Fruit used for firmness evaluation were not used for other destructive analyses. Results were expressed in Newtons (N).

Total Soluble Solids/Titratable Acidity

Five random peaches were selected per replication and treatment to prepare 10 g samples for juice extraction to measure total soluble solids (TSS) and titratable acidity (TA). Samples were homogenized (PowerGen 500, Fisher Scientific, Waltham, MA, USA) and centrifuged for 35 min at 4°C at approximately 4,000× g (Allegra 25R, Beckman Coulter, Brea, CA, USA) to separate the juice from the insoluble material. The supernatant was filtered through cheesecloth, and the samples were then frozen at -20°C until analysis. Total soluble solids (TSS) were measured using a digital pocket refractometer (PAL-1; Atago, Tokyo, Japan) at 20°C and expressed as °Brix. Titratable acidity (TA) was determined by titrating a known volume of juice with 0.1 M NaOH to an endpoint of pH 8.2 using an auto-titrator (DL15, Mettler Toledo,

Greifensee, Switzerland) and expressed as percent malic acid (w/v). The TSS/TA ratio was then calculated by dividing the TSS (°Brix) by the TA values, as explained below.

$$TSS/TA\ ratio = (TSS(°\text{Brix}))/(TA\ (percentage\ of\ acid\ w/v))$$

3. Data analysis

The experiment followed a randomized complete design, with ozone treatment and storage duration as experimental factors. Treatment effects were evaluated independently at each storage time. For each cultivar and sampling day, data were analyzed using one-way ANOVA (Analysis of Variance) with treatment as the fixed effect. The Tukey HSD (Honest Significant Difference) test was used to determine the differences between means, using a 95% confidence interval ($P \leq 0.05$). For decay incidence, data consisted of binary outcomes (decayed vs. non-decayed) with low event frequencies, and thus, treatment effects were evaluated by comparing each O_3 treatment against the control using contingency tables. Fisher's exact test was applied, ensuring appropriate statistical inference for sparse data. All statistical analyses were conducted using RStudio (Posit, PBC, Boston, MA, USA). Prior to statistical analysis, Shapiro-Wilk tests were used to evaluate normality and homogeneity of variance. Firmness data for the 'O'Henry' cultivar at 21 and 28 d of storage and for the 'August Lady' cultivar at 21 d required transformation to meet model assumptions. Square-root and log transformations were applied as appropriate based on residual diagnostics. All other variables and storage times were analyzed using untransformed data. Data preprocessing and transformations were performed using Python (version 3.9; pandas, numpy), and subsequent statistical analyses were conducted as described.

4. Results

4.1. Firmness

Firmness values for both 'O'Henry' and 'August Lady' peaches gradually declined over time until day 14 (Table 1). However, by day 21 of storage, peaches from all treatments, including the control

(CS) and those exposed to 0.5 and 1 ppm of gaseous O_3, demonstrated an unusually increased firmness compared to the 7 and 14 d measurements (Table 1). After 28 d of storage, 'O'Henry' peaches from the control group (CS) had significantly higher firmness values compared to those treated with O_3 (Table 1), whereas 'August Lady' peaches treated with 0.5 ppm O_3 exhibited significantly higher firmness compared to CS and 1 ppm O_3 (Table 1).

4.2. Total Soluble Solids

Total soluble solids (TSS) content remained stable throughout storage for both 'O'Henry' and 'August Lady' peaches, with no significant differences observed among treatments at any given time point evaluated during the 28 d of storage (Table 1). In 'O'Henry' peaches, TSS values ranged from 12.6 to 14.8°Brix, whereas 'August Lady' peaches exhibited TSS values between 11.7 and 14.3°Brix.

4.3. Titratable Acidity

The titratable acidity (TA), expressed as a percentage of malic acid (w/v), showed variations across treatments and storage periods in 'O'Henry' peaches. In contrast, 'August Lady' peaches showed no significant differences between treatments at any evaluated time point. For 'O'Henry' peaches, TA values ranged from 0.40-0.52% on day 7, followed by a progressive decline over time, reaching the lowest level (0.28%) in fruit treated with 1 ppm O_3 by day 28 (Table 1). A similar trend was observed in 'August Lady' peaches, with TA values decreasing from 0.49-0.52% on day 7 to 0.27-0.37% by day 28. However, no significant differences were observed between treatments at any of the evaluated time points.

4.4 TSS/TA Ratio

The TSS/TA ratio for both 'O'Henry' and 'August Lady' cultivars showed an overall increasing trend over time across all treatments (Table 1). Significant differences in the TSS/TA ratio were observed for 'O'Henry' peaches, depending on the gaseous O_3 concentrations and storage durations. After 7 d of storage, control fruit and those treated with 1 ppm O_3 showed significantly higher TSS/TA ratios than

those treated with 0.5 ppm O_3. By day 28, the TSS/TA ratio increased across all treatments, reaching the highest value (53.0) in peaches treated with 1 ppm O_3, followed by 41.2 in 0.5 ppm O_3-treated fruit and 35.2 in control (Table 1). No statistically significant differences between treatments were observed at any evaluation point in the 'August Lady' cultivar. However, TSS/TA ratio values increased gradually during 28 d of storage in all 'August Lady' fruit (Table 1).

4.4. Respiration Rate

The respiration rate (RR) of 'O'Henry' and 'August Lady' peaches generally increased over time, as shown in Table 1. Although some fluctuations were observed throughout storage, no statistically significant differences between treatments were found at any time point, except at the end of storage (day 28), where 'O'Henry' peaches treated with 0.5 ppm gaseous O_3 had higher respiration rates compared to control (CS) fruit. In contrast, 'August Lady' ozone-treated fruit maintained slightly lower respiration rates than the CS after 28 d of storage; however, no statistical significance was observed between the groups.

4.5. Decay Incidence

Across both cultivars, exposure to 1 ppm gaseous O_3 consistently resulted in increased decay incidence after 21 d of cold storage, and pitting incidence after 14 d of cold storage. However, 0.5 ppm O_3 maintained fruit quality comparable to cold storage controls. Quality responses were generally similar between 'O'Henry' and 'August Lady', with treatment effects becoming more pronounced at later storage times.

In 'O' Henry' peaches, no decay was observed in fruit treated with 0.5 ppm and 1 ppm gaseous ozone (O_3) after 7 and 14 d of storage, while control (CS) fruit exhibited 3.6% decay incidence at both time points with no statistically significant difference (Table 2). After 21 d of storage, no decay was recorded in the CS and 0.5 ppm O_3 treatments while the 1 ppm O_3 treatment showed decay incidence, however, no statistically significant differences were observed. At the end of storage (day 28), CS peaches had no

252 decay, while the other treatments showed symptoms of decay incidence with no statistically significant
253 differences (Table 2).

254 For ‘August Lady’ cultivar, peaches stored under 1 ppm gaseous O_3 showed a decay incidence of 3.6%
255 after 7 d of storage, whereas no decay was observed in the remaining treatments (Table 2). At14 and 21 d
256 of storage, decay was absent across treatments, except for fruit exposed to 1 ppm O_3 on 21 d, which again
257 exhibited incidence; however, no statistical differences were detected (Table 2). At the end of storage
258 (day 28), CS fruit remained free of decay, while both O_3 treatments (0.5 and 1 ppm) exhibited decay
259 incidence of 3.6% and 7.1%, respectively, although these differences were not statistically significant
260 relative to the control (Table 2).

261 ***4.6. Weight Loss***

262 The rate of weight loss increased over time for both peach cultivars during the storage period. For
263 ‘O’Henry’ peaches treated with 1 ppm gaseous ozone (O_3), weight loss showed the lowest values during
264 28 d of storage, ranging from 7.0% on day 7, and gradually increasing to 12.0% by day 28 (Fig. 2). All
265 treatments followed a similar increasing weight loss trend. After 28 d of storage, peaches exposed to 0.5
266 ppm gaseous O_3 experienced the highest weight loss (16.9%) compared to the other treatments (Fig. 2).

267 The ‘August Lady’ cultivar followed a similar pattern to ‘O’Henry,’ with all treatments showing a
268 progressive increase in weight loss up to 28 d (Fig. 3). At the end of the storage (28 d), control (CS) and
269 0.5 ppm gaseous O_3 showed similar weight loss ranges of 18.8% and 18.7% respectively. On the other
270 hand, the 1 ppm gaseous O_3 treatment resulted in a lower weight loss of 12.6% (Fig. 3).

271 ***4.7. Visual Appearance and Pitting Incidence***

272 The visual appearance of both ‘O’Henry’ and ‘August Lady’ peaches was affected depending on
273 the ozone (O_3) concentration applied during storage. No pitting incidence was observed in either
274 ‘O’Henry’ or ‘August Lady’ peaches among all treatments during the first 7 d of storage (Figs. 4 and5).
275 After 14 d of storage, intense pitting incidence was detected for both cultivars treated with 1 ppm gaseous

O_3, which intensified as storage time progressed (Figs. 4 and5). On the other hand, the visual appearance of both cultivars treated with 0.5 ppm gaseous O_3 and cold storage (CS) remained unaffected at all evaluation points (Figs. 4 and 5).

4.8. Color Measurements

The skin color values for both cultivars at 7, 14, and 21 d were similar, with no statistically significant differences observed between treatments. At 28 d of storage, color values of 'O'Henry' peaches showed no significant differences between treatments, except for those exposed to 0.5 ppm O_3, which exhibited a significant decrease in C* values compared to the control (CS) fruit (Supplementary). For the 'August Lady' cultivar, no statistically significant differences were observed across all treatments for L*, a*, b*, C*, and h* values.

5. Discussion

This study evaluated the effects of continuous gaseous ozone (O_3) exposure (0.5 and 1.0 ppm) on the postharvest quality attributes of 'O'Henry' and 'August Lady,' two melting flesh, freestone peach cultivars, during 28 d of cold storage at 1.1 °C with 90–95% RH. Fruit from both cultivars softened during the first 14 d across all three treatments, consistent with normal ripening-related softening in climacteric peaches (Franzoni et al. 2023; Xu et al. 2023). In peaches, prolonged cold storage may induce abnormal softening characterized by a transition from juicy to dry and mealy flesh, a disorder linked to climacteric ripening processes regulated by ethylene, which drives cell wall disassembly and texture modification during storage (Arefi et al. 2015; Tucker et al. 2017; Zhang et al. 2022). Consistent with this physiological background, our results showed that after 21 and 28 d of storage, fruit from both cultivars exhibited an abnormal increase in firmness across all treatments, accompanied by shrinkage, dehydration, and a wrinkled outer surface, symptoms of chilling injury development, particularly the leatheriness disorder in peaches that has been previously described by Lurie and Crisosto (2005). Similar increases in firmness after extended cold storage have been reported by McGlasson et al. (2005), Cano-Salazar et al. (2012), and Ceccarelli et al. (2019), indicating that prolonged exposure to low temperatures can result in a leathery texture associated with increased firmness (Brummell et al. 2004). This abnormal increase in firmness can be mechanistically explained by chilling injury-induced cellular dysfunction, which is a major postharvest constraint in peaches and leading to end-of-market quality loss and consumer complaints (Shan et al. 2016; Manganaris et al. 2022). Chilling injury primarily affects cell membrane structure and lipid composition, which disrupts the peach cell membrane integrity and increase the susceptibility of the fruit to oxidative damage (Jin et al. 2014; Lado et al. 2019; Franzoni et al. 2023). These membrane alterations will impair normal ripening processes, restrict water mobility in the cellular level, and reduce tissue flexibility, which ultimately resulting in weakened cellular integrity and the development of firm, leathery texture (Ma et al. 2020). In the present study, continuous gaseous ozone applications at 0.5 and 1 ppm did not alleviate nor intensify chilling injury symptoms, as all treatments

311 exhibited similar internal quality responses during cold storage. This indicates that, under the tested
312 conditions, O_3 exposure did not modify the development or severity of chilling injury in either cultivar.

313 Our results also showed that the addition of 0.5 ppm continuous gaseous O_3 was consistently
314 associated with higher weight loss for both cultivars throughout the 28 d of storage period. In contrast,
315 peaches treated with 1 ppm gaseous O_3 showed lower weight loss compared with the other treatments.
316 While studies on gaseous O_3 applications on peaches are limited, Palou et al. (2002) reported that peaches
317 treated with continuous gaseous O_3 concentrations of 0.3 ppm exhibited increased water loss after five
318 weeks of storage. Another recent study by Buluc and Koyuncu (2022) on pomegranates, stated that
319 gaseous O_3 applications of 3 ppm had a negative impact on the stomatal permeability and degenerated
320 pomegranate skin, resulting in high weight loss during the first month of storage. Additionally, a study on
321 tomatoes by Borland et al. (2007) reported that continuous gaseous O_3 applications of 1 ppm for 6 days
322 resulted in increased weight loss during storage. However, in our study, the reduced weight loss observed
323 at 1 ppm O_3 suggests a different physiological response in peaches, potentially associated with O_3-
324 induced alterations in epidermal structure and gas exchange. Ozone exposure has been shown to affect
325 enzymes related to cell wall integrity such as pectin methylesterase (PME), which is responsible for cell
326 wall structure and surface permeability modifications (Rico et al. 2006; Rodoni et al. 2010). Afsah-Hejri
327 et al. (2021) reported that O_3 treatment altered stomatal function and epicuticular wax structure in figs,
328 which was associated with reduced transpiration rates. Together, these findings support the interpretation
329 that exposure to 1 ppm continuous gaseous O_3 concentration may induce epidermal and cellular damage
330 in peaches, resulting in restricted water vapor diffusion and lower weight loss.

331 Despite some minor fluctuations, the O_3 treatments did not significantly alter total soluble solids
332 (TSS) and titratable acidity (TA). However, both cultivars exhibited a general decline in TA over time. Jia
333 et al. (2020) reported that both ozone-treated and non-treated peaches exhibited a consistent decrease in
334 TA throughout the storage period. Similarly, Ali et al. (2014) and Li et al. (2017) observed that trend in
335 papaya and strawberry, where TA values declined over time. In contrast, both ‘O’Henry’ and ‘August

Lady' cultivars showed a progressive increase in the TSS/TA ratio over the storage duration, reflecting the combined effect of acid depletion and relatively stable sugar levels (Zheng et al. 2021). This trend aligns with the typical ripening behavior of climacteric fruits, where the sugar-to-acid ratio increases as storage progresses(Ali et al. 2021; Khatri et al. 2021; Sati et al. 2023). Neither 0.5 nor 1 ppm gaseous O_3 concentrations had a major significant effect on peach skin color. This parameter remained unaffected by gaseous O_3 treatments for both 'O'Henry' and 'August Lady' peaches. Similarly, Jia et al. (2020) reported that peaches treated with O_3 showed no significant differences in the color values of the cut surfaces after 30 d of storage.

Decay incidence showed a similar pattern for both cultivars over 28 d of storage. For both cultivars and gaseous O_3 treatment the decay incidence was between 3.6 and 7.1% Furthermore, the continuous gaseous O_3 treatment of 1 ppm resulted in a notable visible increase in pitting incidence on both cultivars. Pitting incidence was detrimental to the outer appearance of the peach, which could lead to a significant decrease in fruit marketability. The results of this study indicated that pitting incidence and chilling injury (CI) in peaches were intensified by prolonged storage in combination with continuous exposure to 1 ppm gaseous O_3, indicating an interactive effect between storage duration and continuous O_3 treatment. It has been reported that gaseous O_3 in combination with CI, can lead to disrupted cellular integrity, membrane damage, and accelerated tissue degradation, potentially manifesting as pitting on the surface (Valenzuela et al. 2017). Ozone strong oxidative agent, and prolonged exposure to low gaseous concentrations during storage has been reported to induce oxidative stress responses in fruits, depending on cultivar sensitivity and exposure duration (Forney et al. 2003; Cayuela et al. 2009). When O_3 comes into contact with fruit surfaces, it promotes the generation of ROS, including, hydroxyl radicals (·OH), and excessive accumulation of these species can be toxic to plants or fruits tissues (Sarron et al. 2021; Li et al. 2022; Piechowiak et al. 2022). Several studies have confirmed that elevated ROS levels are closely associated with oxidative stress-induced damage to cellular membranes and structural components in

horticultural commodities (Vainonen and Kangasjärvi 2015; Valenzuela et al. 2017; Ma et al. 2022; Drogoudi et al. 2023).

The effects of gaseous O_3 application vary significantly among fruits and vegetables due to differences in physiological characteristics and tolerance to this compound, resulting in variable impacts on postharvest quality attributes. Recent studies on cantaloupes, strawberries, mandarins, and mangoes have reported promising results, demonstrating that O_3 applied at low to moderate concentrations (typically ranging from aqueous O_3 treatments to gaseous O_3 concentrations between approximately 0.3 and 5 ppm, depending on the commodity and application method) can effectively suppress fruit decay and extend shelf life(Contigiani et al. 2018; Chen et al. 2020; Maryam et al. 2021; Bambalele et al. 2023; Lemic et al. 2024; Peng et al. 2024). However, the results of this study, together with our previous findings (Giannopoulos et al. 2025b), demonstrated that continuous exposure to gaseous O_3 at concentrations ranging from 0.5 to 5 ppm is detrimental to peach quality. These results indicate a high sensitivity of the evaluated cultivars to gaseous O_3, making them more susceptible to decay and weight loss. Consistent with these observations, recent studieshave also shown that O_3 treatments can cause severe tissue damage, followed by weight loss and skin tissue burning (Juhnevica-Radenkova et al. 2019; Giannopoulos et al. 2025a). Therefore, future studies should focus on a broader range of cultivars, as well as O_3 doses, and most importantly, different application techniques such as intermittent or pulsed exposure. Another future approach could also incorporate biochemical markers of oxidative damage and chilling injury to better define safe and effective O_3 application thresholds for commercial peach storage.

Disclosure of interest

The authors report there are no competing interests to declare.

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576 **Tables**

Table 1. Physiochemical characteristics of 'O'Henry' and 'August Lady' peaches in cold storage and/or gaseous ozone treatment: Firmness, Total soluble solids (TSS), Titratable acidity (TA), TSS/TA Ratio, and Respiration rate (RR).

Variety	Day	Treatments	Firmness (N)	TSS (°Brix)	TA (% malic acid, w/v)	TSS-Acid Ratio	RR (mL CO_2 kg^{-1} h^{-1})
'O' Henry'	7	CS	3.35 ± 0.29 **a**	14.1 ± 0.10 **a**	0.45 ± 0.06 **b**	31.8 ± 1.28 **a**	39.8 ± 0.89 **a**
		0.5 ppm O_3	3.35 ± 0.28 **a**	12.6 ± 0.44 **a**	0.52 ± 0.02 **a**	24.2 ± 1.19 **b**	39.9 ± 1.42 **a**
		1 ppm O_3	3.31 ± 0.27 **a**	13.0 ± 0.41 **a**	0.40 ± 0.01 **b**	31.9 ± 0.52 **a**	40.9 ± 0.79 **a**
	14	CS	2.78 ± 0.27 **a**	14.8 ± 0.13 **a**	0.53 ± 0.06 **a**	27.8 ± 0.09 **a**	51.2 ± 6.83 **a**
		0.5 ppm O_3	3.18 ± 0.29 **a**	14.7 ± 0.62 **a**	0.39 ± 0.04 **b**	38.3 ± 5.98 **a**	60.9 ± 0.97 **a**
		1 ppm O_3	3.17 ± 0.17 **a**	13.4 ± 0.10 **a**	0.44 ± 0.00 **ab**	30.4 ± 0.22 **a**	56.6 ± 1.51 **a**
	21	CS	5.55 ± 0.12 **a**	14.2 ± 0.37 **a**	0.47 ± 0.05 **a**	30.8 ± 4.31 **a**	54.0 ± 1.12 **a**
		0.5 ppm O_3	4.76 ± 0.12 **a**	14.3 ± 0.18 **a**	0.42 ± 0.04 **a**	34.7 ± 2.93 **a**	52.3 ± 1.96 **a**
		1 ppm O_3	4.25 ± 0.19 **a**	13.0 ± 0.23 **a**	0.38 ± 0.03 **a**	33.8 ± 0.88 **a**	53.4 ± 1.53 **a**
	28	CS	12.4 ± 0.13 **a**	13.7 ± 0.68 **a**	0.40 ± 0.03 **a**	35.2 ± 4.90 **b**	53.7 ± 0.82 **b**
		0.5 ppm O_3	7.02 ± 0.21 **b**	14.7 ± 0.44 **a**	0.36 ± 0.02 **ab**	41.2 ± 2.66 **ab**	59.4 ± 0.23 **a**
		1 ppm O_3	5.37 ± 0.17 **b**	14.8 ± 0.41 **a**	0.28 ± 0.05 **b**	53.0 ± 2.44 **a**	55.5 ± 2.02 **ab**
'August Lady'	7	CS	3.38 ± 0.25 **a**	14.3 ± 0.17 **a**	0.54 ± 0.06 **a**	27.3 ± 3.29 **a**	38.6 ± 1.71 **a**
		0.5 ppm O_3	3.12 ± 0.15 **a**	14.0 ± 0.44 **a**	0.49 ± 0.02 **a**	28.5 ± 1.99 **a**	36.3 ± 1.31 **a**
		1 ppm O_3	3.26 ± 0.14 **a**	11.7 ± 0.98 **a**	0.52 ± 0.03 **a**	22.8 ± 2.86 **a**	36.4 ± 2.36 **a**
	14	CS	2.88 ± 0.16 **ab**	14.3 ± 0.13 **a**	0.48 ± 0.01 **a**	29.9 ± 0.78 **a**	46.0 ± 3.88 **a**
		0.5 ppm O_3	2.56 ± 0.14 **b**	14.3 ± 0.52 **a**	0.47 ± 0.02 **a**	30.6 ± 2.47 **a**	50.9 ± 0.75 **a**
		1 ppm O_3	3.37 ± 0.24 **a**	12.9 ± 0.76 **a**	0.42 ± 0.03 **a**	31.1 ± 3.60 **a**	40.7 ± 3.46 **a**
	21	CS	8.17 ± 0.12 **a**	13.3 ± 0.36 **a**	0.45 ± 0.01 **a**	29.6 ± 1.14 **a**	51.8 ± 2.01 **a**
		0.5 ppm O_3	10.0 ± 0.11 **a**	13.6 ± 0.48 **a**	0.39 ± 0.02 **a**	35.4 ± 3.31 **a**	59.8 ± 2.18 **a**
		1 ppm O_3	7.26 ± 0.17 **a**	13.9 ± 0.43 **a**	0.40 ± 0.00 **a**	34.1 ± 0.41 **a**	53.2 ± 1.80 **a**
	28	CS	10.9 ± 0.62 **b**	13.3 ± 0.60 **a**	0.37 ± 0.02 **a**	36.3 ± 3.26 **a**	53.1 ± 0.32 **a**
		0.5 ppm O_3	15.5 ± 1.21 **a**	12.7 ± 0.06 **a**	0.27 ± 0.03 **a**	48.0 ± 5.45 **a**	46.9 ± 3.00 **a**
		1 ppm O_3	11.5 ± 1.01 **b**	12.7 ± 0.71 **a**	0.28 ± 0.01 **a**	45.0 ± 0.92 **a**	46.5 ± 1.16 **a**

Note: The study included three treatments: Cold Storage (CS) as the control, and gaseous O_3 applications at 0.5 and 1 ppm concentrations. The table shows the quality parameters: firmness expressed in Newton (N), total soluble solids (TSS)

expressed in (°Brix), titratable acidity (TA) expressed in (% malic acid, w/v), total soluble solids to acid ratio, and respiration rate (RR) expressed in mL CO_2 kg^{-1} h^{-1}. Measurements were taken at 7, 14, 21, and 28 days of storage. The values presented are means ± standard error (SE) from the formula SE = SD / √n. Statistically significant differences among treatments are indicated by different letters (p-value < 0.05).

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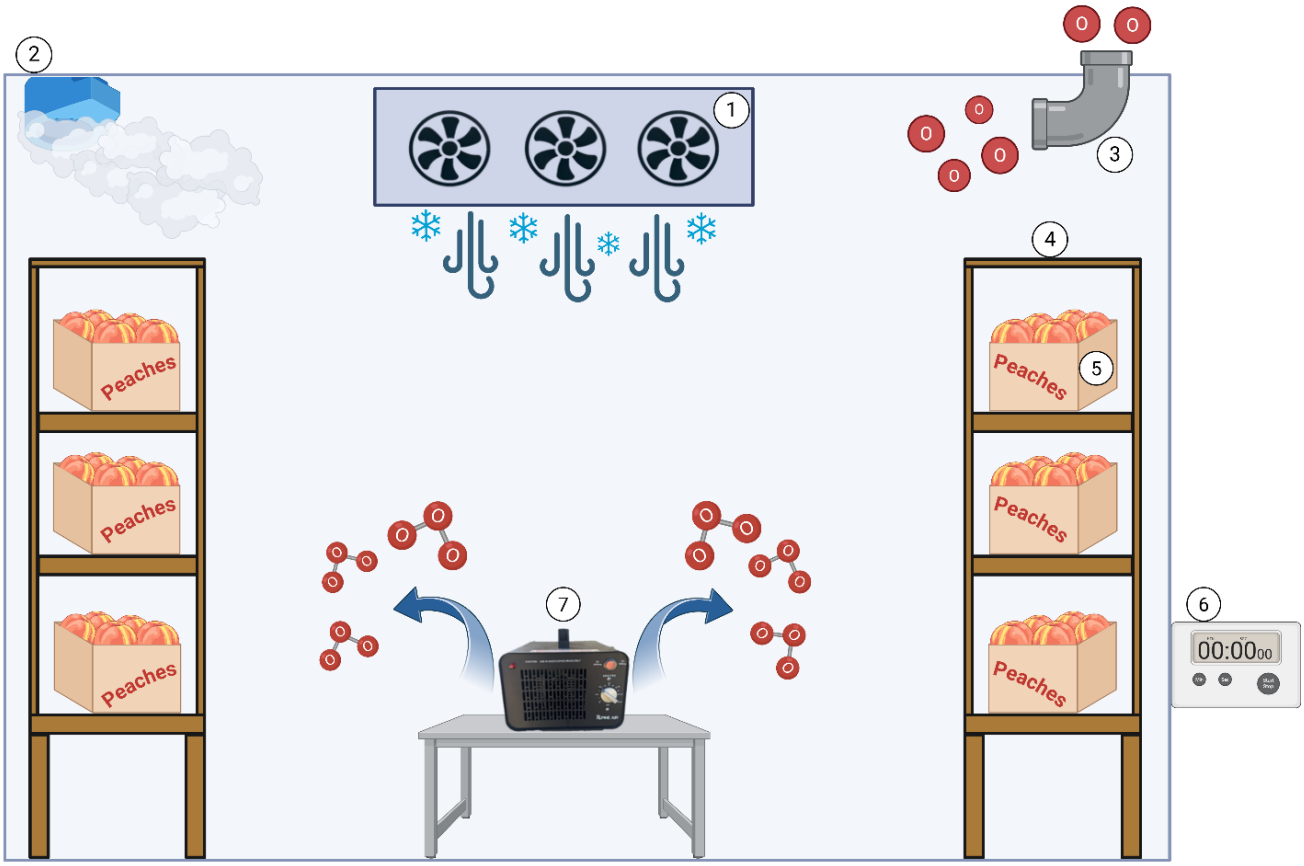
Table 2. Decay Incidence in ‘O’Henry’ and ‘August Lady’ Peaches over 28 days of storage under different conditions

Variety	Day	Treatments	Decay (%)	P-value vs CS
‘O’Henry’	7	CS	3.6	-
		0.5 ppm O_3	0.0	1
		1 ppm O_3	0.0	1
	14	CS	3.6	-
		0.5 ppm O_3	0.0	1
		1 ppm O_3	0.0	1
	21	CS	0.0	-
		0.5 ppm O_3	0.0	1
		1 ppm O_3	7.1	0.49
	28	CS	0.0	-
		0.5 ppm O_3	3.6	1
		1 ppm O_3	7.1	0.49
‘August Lady’	7	CS	0.0	-
		0.5 ppm O_3	0.0	1
		1 ppm O_3	3.6	1
	14	CS	0.0	-
		0.5 ppm O_3	0.0	1
		1 ppm O_3	0.0	1
	21	CS	0.0	-
		0.5 ppm O_3	0.0	1
		1 ppm O_3	3.6	1
	28	CS	0.0	-
		0.5 ppm O_3	3.6	1
		1 ppm O_3	7.1	0.49

Note: Treatments included Cold Storage (CS) as the control and gaseous O_3 applications at concentrations of 0.5 and 1 ppm. Decay incidence was analyzed using 2×2 contingency tables (decayed vs. non-decayed) comparing each ozone treatment with the control within each cultivar and storage day. Expected cell counts were calculated from marginal totals. Because expected counts were < 5 in multiple comparisons, Fisher’s exact test was used.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579 Figure 1.

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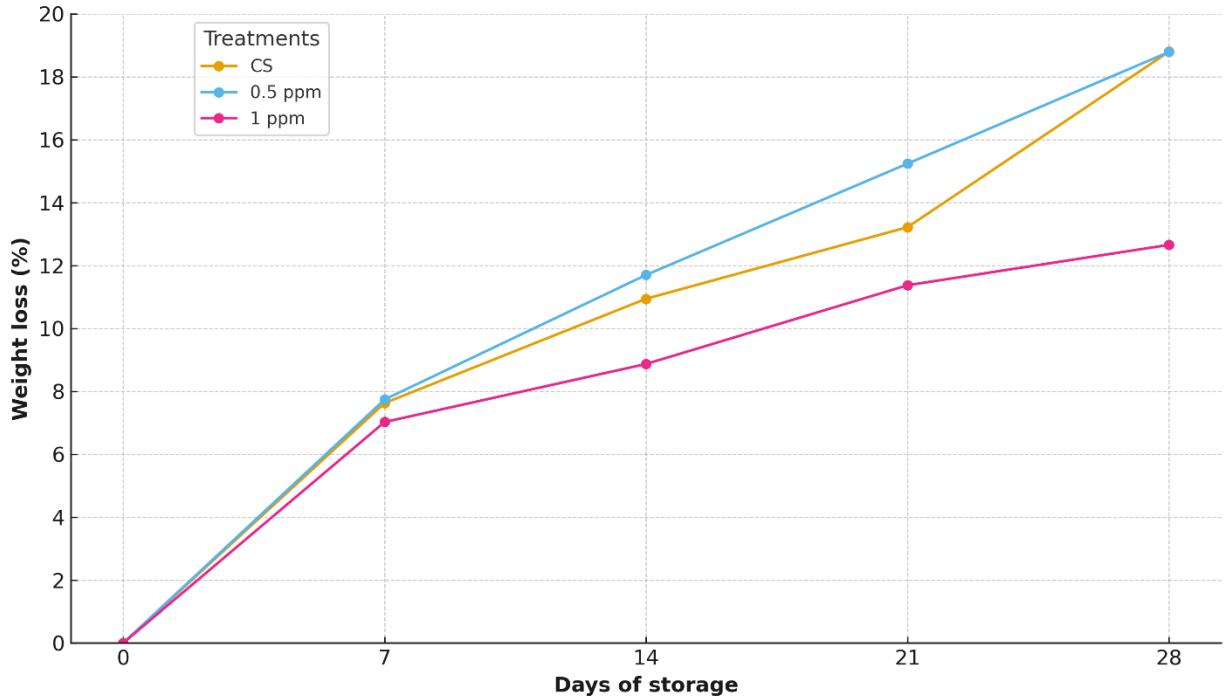
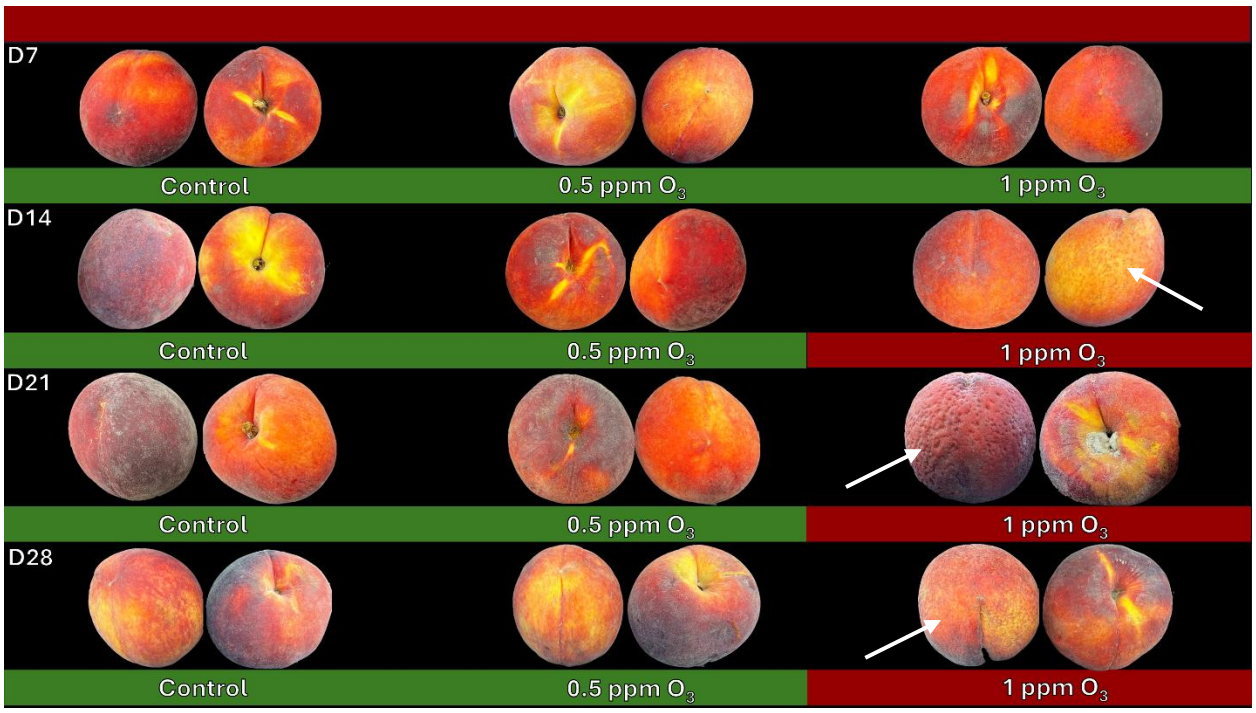


581 Figure 2.

582 Figure 3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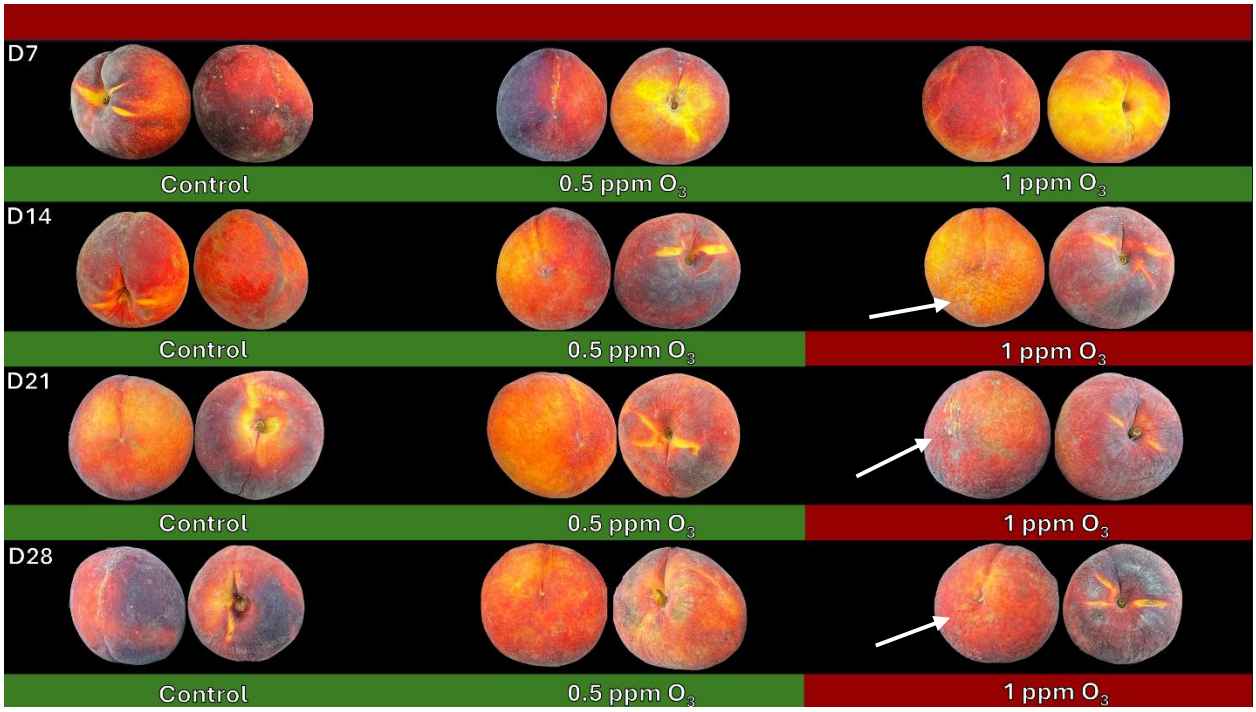
583 Figure 4.



584 Figure 5.

List of Figure Captions

Figure 1. Schematic diagram of a cold storage room including: 1. Refrigeration unit, 2. Humidifier, 3. Air vent, 4. Wooden racks, 5. Fruit boxes, 6. Timer, 7. Gaseous ozone generator. The ozone generator converts atmospheric oxygen (O_2) into ozone (O_3) through a corona discharge mechanism connected with a timer to regulate ozone exposure.

Figure 2. Percentage of weight loss in 'O' Henry' peaches across 28 d of storage. The treatments included cold storage (CS) as control (Orange line), 0.5 ppm O_3 (Blue line), and 1 ppm O_3 (Purple line).

Figure 3. Percentage of weight loss in 'August Lady' peaches across 28 d of storage. The treatments included cold storage (CS) as control (Orange line), 0.5 ppm O_3 (Blue line), and 1 ppm O_3 (Purple line).

Figure 4. Visual appearance of 'O' Henry' over 28 d of cold storage at 1.1 °C and 95% RH. The treatments included Cold Storage as the control and gaseous O_3 applications at concentrations of 0.5 and 1 ppm. In the visual representation, green indicates peaches free of decay or pitting incidence, while red highlights peaches with high decay or pitting incidence, and white arrows indicate pitting incidence.

Figure 5. Visual appearance of 'August Lady' over 28 d of cold storage at 1.1 °C and 95% RH. The treatments included Cold Storage as the control and gaseous O_3 applications at concentrations of 0.5 and 1 ppm. In the visual representation, green indicates peaches free of decay or pitting incidence, while red highlights peaches with high decay or pitting incidence, and white arrows indicate pitting incidence.