



The effect of short-term gaseous ozone treatment on physical attributes and senescence-related enzymes of 'Eureka' lemons during cold sterilization

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ARTICLE INFO

Keywords:

Citrus
Gaseous ozone
Postharvest treatments
Physical attributes
Enzymes
Shelf-life

ABSTRACT

Due to restrictions on the use of synthetic fungicides on fresh produce, there is growing interest in residue-free technologies such as ozone (O₃), which may enhance fruit defence mechanisms against postharvest disorders. This study examined the effect of gaseous O₃ on oxidative stress-related defence enzymes and the physical quality attributes of 'Eureka' lemons during cold sterilization. Fruit was exposed to 0.3 mg/L ozone for 24 or 48 h, then stored at 0 °C as the recommended temperature for sterilization and 95% RH for 28 d, followed by 7 d at ambient temperature. Physicochemical variables, including colour, firmness, lipid peroxidation, catalase (CAT), glutathione reductase (GR), peroxidase (POD) and lipoxygenase (LOX) were assessed bi-weekly. Ozone treatments (48 h and 24 h) exhibited higher decay incidence between day 28 and 35 by 4% and 3.2%, respectively, compared to 0.91% in the untreated fruit. Although the O₃ (48 h) treatment showed higher decay ($p < 0.001$), it had slightly better colour and firmness at the end of the assessment period. Gaseous O₃ significantly increased CAT, LOX, POD, and GR ($p < 0.001$) from 0 to 28 days. A slight decline in CAT and GR was observed during ambient storage, particularly under O₃ (48 h). Overall, these results suggest that O₃ may exacerbate senescence traits of 'Eureka' lemons, and its use may compromise fruit quality and promote decay, particularly during cold sterilization. Considering that CAT and GR were significantly increased, further research should explore the shorter O₃ durations and their impact on decay-causing pathogens and senescence-linked enzymes, specifically POD and LOX, to enhance the efficacy and safety of ozone as a postharvest treatment for citrus fruit.

1. Introduction

Lemon (*Citrus limon*) is an important fruit due to its extensive applications across various sectors, including the food, medical, pharmacological, and cosmetics industries [1]. This significance is attributed to its abundant bioactive compounds, which include phenolic compounds, carotenoids, vitamins (A, C, and B), minerals, citric acid, and essential oils [2]. Beyond biochemical characteristics, physical quality attributes such as colour, freshness, and firmness are essential for consumer acceptance [3]. Numerous studies have indicated that various factors, including storage conditions and treatment methods, significantly influence the physicochemical properties of citrus fruits [4,5].

Lemons contain various enzymes, including catalase (CAT), peroxidase (POD), lipoxygenase (LOX), and glutathione reductase (GR) that protect against oxidative stress. These enzymes associated with

senescence in lemons are critical for maintaining fruit quality and longevity. For instance, LOX contributes to the breakdown of membrane lipids during cold storage, leading to chilling injury and formation of conjugated double bond systems in hydroperoxides [6]. Peroxidase is involved in detoxifying harmful substances, regulating hormones, and generating reactive oxygen species (ROS) [7]. Additionally, it promotes lignification of cell walls, forming a barrier against pathogen invasion and aiding tissue repair post-injury [8]. Catalase acts as a vital antioxidant, safeguarding the fruit from oxidative stress associated with H₂O₂ accumulation during ripening [9]. Glutathione reductase is essential for maintaining cell membrane integrity and preventing lipid peroxidation during chilling stress [10].

In recent years, research has demonstrated benefits in utilizing ozone (O₃) due to its self-decomposing nature and potent oxidative properties [11–13]. Ozone functions as a powerful disinfectant owing to its

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<https://doi.org/10.1016/j.jafr.2026.102788>

Received 8 December 2025; Received in revised form 9 February 2026; Accepted 21 February 2026

Available online 26 February 2026

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triatomic oxygen structure. Several studies have been conducted on the effect of ozone on certain fresh produce. For instance, Wang et al. [14] studied how gaseous ozone treatment prolonged the shelf-life of fresh-cut kiwifruit by maintaining its ascorbic acid content. Recently, Bambalele et al. [15] evaluated the antifungal activity of gaseous ozone against stem-end rot of 'Keitt' mango fruit, and Gutarowska et al. [16] studied the effectiveness of ozonation for improving the microbiological safety of fresh-cut parsley.

Ozone demonstrates significant disinfectant capabilities and can effectively inactivate pathogenic bacteria such as *Escherichia coli*, *Salmonella typhimurium*, and *Listeria monocytogenes*, as well as viruses and protozoa [17,18]. According to Xue et al. [19] O₃ can react with pesticide residues in grains [12] and pollutants in wastewater [20], owing to its high oxidizing capacity. Importantly, unlike many postharvest treatments, O₃ does not leave harmful residues on treated produce. However, despite extensive studies on ozone use in fresh fruits and vegetables, little is known about its impact on physicochemical quality attributes in citrus, particularly lemons.

Citrus fruit destined to the export markets, such as the USA, Japan, or Europe, undergo cold sterilization, which is a mandatory phytosanitary treatment. Typically beginning at the packhouse and continuing during the sea freight, the fruit is kept at -0.6 to 0 °C for 12 to 28 days, depending on the importing country's regulations. However, during cold sterilization, citrus fruit tends to lose quality and develop storage disorders due to the aggressive nature of the treatment. Various postharvest treatments, such as edible coatings and methyl jasmonate, have been shown to retain citrus fruit quality during cold sterilization. As mentioned above, gaseous ozone treatment can improve the defense mechanism, including increased ascorbic acid content, in treated produce [14]. Although ozone has been previously investigated on citrus fruit, there is very limited information reporting its impact on physicochemical quality and senescence-related enzymes in lemon fruit. Therefore, this study investigated the effects of O₃ on key physical quality properties and senescence-related enzymes in 'Eureka' lemons, particularly for the retention of quality during cold sterilization.

2. Materials and methods

2.1. Fruit material

Lemon fruit (cv. 'Eureka') were procured from Carisbrooke Citrus Valley Farm, Ixopo, KwaZulu-Natal (30.24172° S, 30.01315° E) and transported to the postharvest Laboratory at the University of KwaZulu-Natal. Upon arrival at the Postharvest Laboratory, fruit were graded for colour and size uniformity and stored in carton boxes. The experiment followed a completely randomised design, in which five replicates (22 fruit per replicate) were used for mass loss, colour, firmness, and decay rate. For CAT, POD, LOX, GR, and lipid peroxidation analyses, three replicates (22 fruits per replicate) were used.

2.2. Ozone treatment and cold storage

The fruit were treated with 0.3 mg/L O₃ equipped with an ozone analyzer (Corona discharge ozone generator, Ozone Purification Technology, Johannesburg, South Africa). Fruit were assigned to respective gaseous ozone treatments where time was the treatment factor: T1 = Control (untreated lemons); T2 = 24 h; T3 = 48 h. After treatment, fruit were cold stored at 0 °C, 95% RH for 28 d as per the cold sterilization protocol for most export markets. Fruit were then exposed to ambient temperatures (16 °C) for 7 d. Sampling was done bi-weekly (D₀, D₁₄, D₂₈) and on D₃₅.

2.3. Sample preparation

Flavedo (peel) was excised and immediately stored at -22 °C. Samples were thereafter freeze-dried using a VirTis BenchTop Pro

freeze-drier (SP Scientific, Warminster, PA, USA), pulverised into powder, and stored at -22 °C until use.

2.4. Decay incidence

Decay incidence was quantified using the methodology outlined by Yang et al. [21] using Eq. (1).

$$\text{Decay incidence} = \frac{\text{Number of decayed fruits}}{\text{Total number of fruits}} \times 100 \quad \text{Eq. 1}$$

2.5. Mass loss

The measurement of fruit mass was conducted using a calibrated electronic weighing scale (RADWAG Wagi Electronic Inc., Poland). Lemons were weighed upon arrival at the laboratory (Day zero) and at the end of each storage interval, employing a separate sample of five replicates for each treatment condition. Mass loss was calculated using Eq. 2

$$\text{Mass loss (\%)} = \frac{M_i - M_f}{M_i} \times 100$$

Where, M_i = Initial mass

M_f = Final mass

Eq. 2

2.6. Fruit colour

Fruit colour was objectively quantified based on L*, a*, b* and h parameters using CR 400 Chromameter (Minolta Co. Ltd., Osaka, Japan). Measurements were taken at three random equatorial position of 6Y = 91.59, X = 0.3167 and y = 0.3315 per fruit. The total colour difference was expressed as a citrus colour index (CCI) (Eq. (3)).

$$CCI = \frac{1000 \times a}{L \times b} \quad \text{Eq. 3}$$

2.7. Fruit firmness

Fruit firmness was quantified according to the protocol established by González-Aguilar et al. [22] utilizing a handheld penetrometer (Bareiss, Germany) equipped with an 8 mm plunger tip. The firmness measurements were taken on opposite cheeks of each fruit, and the resulting data were expressed in newtons (N).

2.8. Determination of catalase

Catalase (EC 1.11.1.6) activity was assessed at 25 °C according to Kato and Shimizu [23]. The enzyme assay comprised 3.125 mM hydrogen peroxide (H₂O₂) in a 50 mM phosphate buffer at pH 7.0, combined with 200 µL of the enzyme extract, resulting in a total assay volume of 3 mL. Catalase activity was determined spectrophotometrically by measuring the decrease in absorbance of H₂O₂ at 240 nm, and the results were expressed as nanomoles of H₂O₂ decomposed per milligram of protein per min (nmol H₂O₂ mg protein⁻¹ min⁻¹).

2.9. Determination of peroxidase activity

Peroxidase activity was performed following Criado et al. [24] with slight modifications. In triplicate, 1 g of fruit sample was homogenized in 10 mL of sodium phosphate buffer (0.2 M, pH 7.0). The homogenate was then centrifuged at 15,000 rpm for 15 min at 4 °C using an Avanti J-265 XP centrifuge (Beckman Coulter, Indianapolis, USA). Subsequently, 0.1 mL of the enzyme extract was mixed with 2.7 mL of sodium phosphate buffer (0.05 M). Following this, 0.1 mL of H₂O₂ (1.5% w/v) and 0.2 mL of 1% p-phenylenediamine were added to the mixture. The absorbance was measured at 485 nm using a spectrophotometer

(Shimadzu Scientific Instruments INC., Columbia, USA). Enzyme activity was quantified as the increase in absorbance at 485 nm per milligram of protein per min.

2.10. Determination of lipoxygenase

Lipoxygenase (EC 1.13.11.12) was extracted using the method described by Rue et al. [25], with minor modifications to ensure enzyme stability. Protein extraction was conducted at 4 °C to minimize degradation. Flavedo powder (5 g dry weight) was homogenized in 5 mL of a 50 mM Tris-HCl buffer (pH 8), supplemented with 10 mM KCl, 500 mM sucrose, and 0.5 mM phenylmethylsulfonyl fluoride, all prepared at 4 °C. The mixture was centrifuged at 12,000 rpm for 10 min at 4 °C using an Avanti J-265 XP centrifuge (Beckman Coulter, Indianapolis, USA). The supernatants obtained were utilized for the assessment of LOX activity.

Lipoxygenase activity was quantified by preparing a standard assay mixture consisting of 200 µL Tween 20 and 40 µL linoleic acid in 40 mL of 0.1 mM phosphate buffer (pH 7.0). The reaction was initiated by adding 0.2 mL of the enzyme extract to 1 mL of the standard assay mixture. Absorbance was measured spectrophotometrically (Shimadzu Scientific Instruments INC., Columbia, USA) at 234 nm at room temperature (approximately 16 °C). One unit of LOX activity was defined as the quantity of enzyme that produces an increase in absorbance at 234 nm of 0.01 min⁻¹. The protein concentration of the enzyme extract was determined using the Bradford assay [26], and LOX activity was expressed as units per milligram of protein.

2.11. Determination of glutathione reductase (GR)

Glutathione reductase extraction was performed according to the method described by Sala and Lafuente [27], with minor modifications. Flavedo powder (1 g dry weight) was homogenized in 10 mL of a 100 mM potassium phosphate buffer (pH 7.5) containing 0.5 mM EDTA, and the mixture was maintained at 4 °C to prevent enzyme degradation. The solution was then centrifuged at 27,000 rpm for 15 min at 4 °C using an Avanti J-265 XP centrifuge (Beckman Coulter, Indianapolis, USA), and the resulting supernatant was utilized for the assessment of GR activity as per the protocol established by Smith et al. [28]. One unit of GR activity was defined as the amount of enzyme that catalyzes the oxidation of 1 µmol of NADPH per min. The protein concentration in the enzyme extract was determined using standard methods.

2.12. Determination of lipid peroxidation

The level of lipid peroxidation in lemon flavedo was quantified following the methodology described by Chong et al. [29]. A sample of lemon flavedo tissue (0.2 g dry weight) was homogenized in 4 mL of 0.1% (w/v) trichloroacetic acid (TCA) using a pre-chilled mortar and pestle at 4 °C. The homogenate was then centrifuged at 3000 rpm using an Avanti J-265 XP centrifuge (Beckman Coulter, Indianapolis, USA) for 5 min. Subsequently, 10 mL of the supernatant was transferred to a test tube containing a solution of 20% (w/v) TCA, 0.01% (w/v) butylated hydroxytoluene, and 0.65% (w/v) thiobarbituric acid. The samples were mixed vigorously and incubated at 95 °C for 30 min before cooling to 4 °C and centrifuged again at 3000 rpm for 10 min. The absorbance of the resulting solution was measured at both 532 nm and 600 nm using spectrophotometer (Shimadzu Scientific Instruments INC., Columbia, USA). Total malondialdehyde (MDA) equivalents were calculated and expressed as total MDA (nmol g⁻¹ dry weight) according to the methods outlined by Siboza and Bertling [30].

2.13. Statistical analysis

Statistical analyses were conducted with GenStat statistical software (GenStat 23rd Edition, VSN International Ltd, UK). Analysis of variance was performed, and treatment means were compared using Tukey's least

significant difference at a significance level of 5%. Microsoft Excel (version 11) was used to plot the graphs.

3. Results and discussion

3.1. Decay incidence

The interaction between treatment and storage time had no significant ($p > 0.05$) effect on decay incidence of 'Eureka' lemons (Fig. 1). Similarly, storage duration had no significant effect on decay incidence. Untreated fruit showed a relatively low decay incidence of 0.91% at day 35, while treated fruit showed a high decay incidence according to the intensity of the treatment, with O₃ (48 h) showing the highest decay incidence of 6.49%.

Untreated fruit first showed signs of decay at Day 35, whereas O₃ treated fruit began decaying earlier at day 28. Between Day 28 and Day 35, decay increased to 4% in O₃ (48 h), exhibiting the highest, 3.2 % in O₃ (24 h) and 0.91% in the control (Fig. 1). These findings align with those of Cao et al. [31] who found that 'Guichang' kiwi fruit treated with 300 mg/L for 3 h, stored at 1 °C and 85 ± 5% RH for 120 d had the highest decay incidence compared to those treated with 100 mg/L and 200 mg/L of O₃. Notably, decay incidence was about 2% lower in O₃ (24 h) than O₃ (48 h), suggesting that long O₃ exposure and cold temperatures might compromise the cellular tissues and membrane integrity of the treated fruit, thereby increasing fungal decay incidence. It could further be argued that the high decay incidence observed in ozone-treated fruit was exacerbated by chilling injury, as ozone also induces oxidative stress, which possibly compromised the fruit's tolerance to cold stress. Notably, the fruit decay, particularly in ozone-treated fruit, was preceded by a dry and sunken membrane, which is a sign of rind breakdown in citrus fruit [30]. Ozone can be highly phytotoxic and can trigger ROS synthesis, leading to cellular damage and fungal decay, especially at extremely low temperatures.

In contrast, Nayak et al. [32] found that 'Winter Dawn' strawberry fruits treated with 0.1 mg/L aqueous O₃ for 2 min and stored at ambient conditions (25 ± 2 °C and 45–50% RH) resulted in 22% decrease compared to 44% decrease in untreated fruit. Ozone changes the microbial community on the surface of the fruit, and this could be another factor contributing to the decay incidence. While it may reduce some microorganisms, it can also allow the proliferation of the ozone-resistant organisms, which makes the fruit vulnerable to decay [33]. Lemons are known to have a low pH (acidic) as compared to other fruits. The low pH is instrumental in delaying the onset of decay incidence [34]. According to the study by Panou et al. [35], the observation was that the fresh strawberries (*Carbarosa*) treated with 0.5 mg/L and 1.0 mg/L stored at 1 °C, 90% RH showed an increase in pH and thereby causing decay [36, 37]. The concentration of gaseous ozone and the application period are important factors affecting the decay incidence.

3.2. Fruit mass loss

Statistically, the treatments as well as the interaction between the treatments and storage duration did not significantly ($p > 0.001$) affect the mass loss of the lemon fruit (Fig. 2). However, storage duration significantly affected mass loss. For all the treatments, mass loss sharply increased during cold sterilization and shelf-life. At the end of quality assessments, the control, O₃ (48 h), and O₃ (24 h) treatments had a mass loss of 16%, 10%, and 9%, respectively. The findings of this study are inconsistent with those reported by Li et al. [38], Shen et al. [39], and Lui et al. [40], which demonstrated reduced mass loss in fresh-cut red pitaya, 'Red Delicious' apples, and garlic kept in ozonated cold storage. Ozone treatment has been shown to preserve cellular integrity, minimizing the mass loss through preventing the leakage of water through lenticels [39,40]. The marginal impact of gaseous ozone on mass loss observed in this study could be partly linked to its phytotoxicity, which resulted in stress-induced leakage of cellular components across the

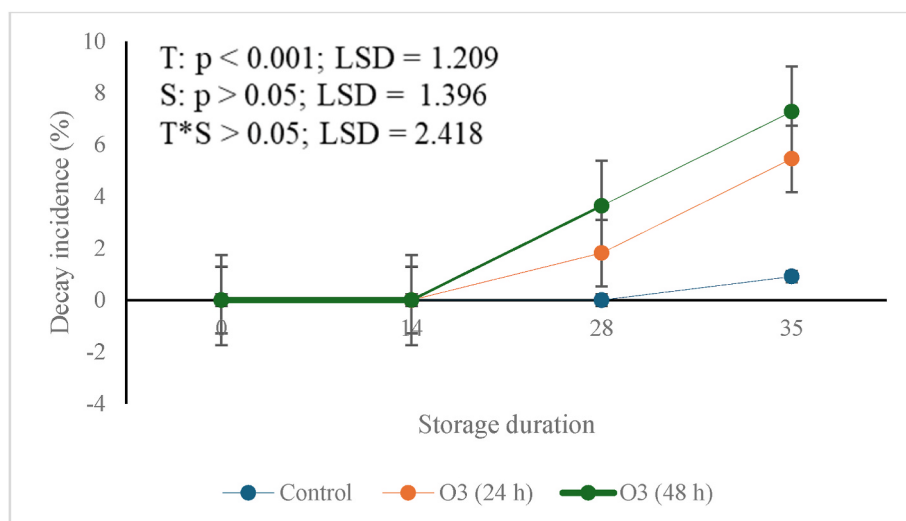


Fig. 1. Effect of gaseous ozone on the decay rate of 'Eureka' lemons during cold sterilization at 0 °C for 28 days and shelf-life at ambient temperature for seven days ($\pm$ SE, $n = 5$).

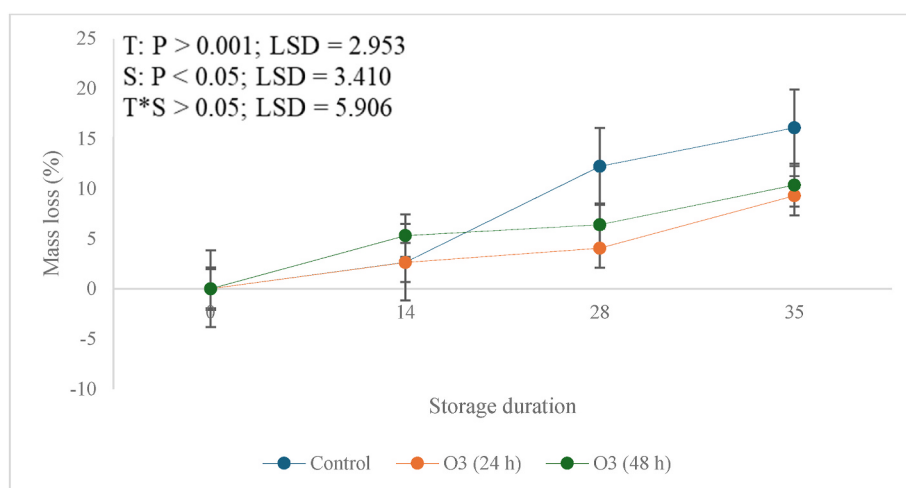


Fig. 2. Effect of gaseous ozone on the mass loss of 'Eureka' lemons during cold sterilization at 0 °C for 28 days and shelf-life at ambient temperature for seven days ($\pm$ SE, $n = 5$).

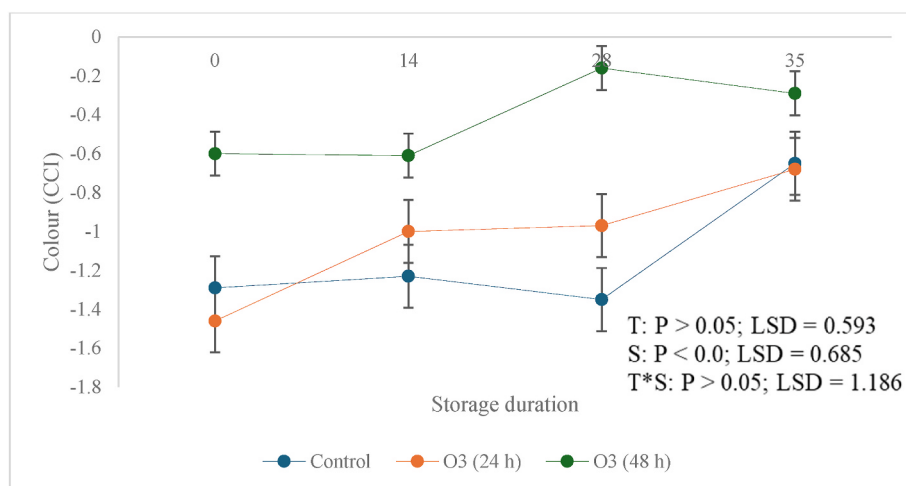


Fig. 3. Effect of gaseous ozone on colour (CCI) of 'Eureka' lemons during sterilization at 0 °C for 28 days and shelf-life at ambient temperature for seven days ($\pm$ SE, $n = 5$). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

rind.

3.3. Colour

Biochemical mechanisms coordinate the changes in fruit coloration. Ozone exposure has been shown to enhance the levels of antioxidants in fruits, notably phenolic compounds and carotenoids, which are crucial for the pigmentation of lemon fruits [38]. The oxidative stress induced by O_3 can stimulate the synthesis of secondary metabolites that significantly contribute to fruit pigmentation [38].

In this study, the gaseous ozone treatment and interaction between treatment and storage did not significantly ($p > 0.05$) affect fruit colour. However, storage duration significantly ($p < 0.05$) influenced fruit colour changes. Untreated fruit showed variations in colour as the values fluctuated throughout storage (Fig. 3). At the beginning of the storage (Day 0) the control group showed CCI of -1.29 with a sharp 57% decline between Day 14 and Day 28. In contrast, O_3 (24 h) treated fruit showed consistent increase in CCI during storage, while O_3 (48 h) treated fruit followed a similar trend until Day 28, after which a decline of 27% was recorded. These findings are in contrast with Bambalele et al. [15], who reported higher chroma values in 'Keitt' mango fruit exposed to different durations of O_3 treatment. This suggests that exposure time, storage duration, and temperature are critical when developing ozonated storage protocols. The insignificant impact of gaseous ozone observed in this study could be due to the excessive oxidative stress imposed by cold sterilization (0°C , 95% RH), which favoured the synthesis of ROS over secondary metabolites such as carotenoids and anthocyanins.

3.4. Firmness

In this study, the interaction between treatment and storage duration did not significantly ($p > 0.05$) affect fruit firmness. Notably, the treatments significantly ($p < 0.001$) affected the firmness level during storage. For all the O_3 treatments, there was a significant decline in fruit firmness (Fig. 4). This study demonstrated that 'Eureka' lemon subjected to O_3 (48 h) treatment exhibited the lowest firmness loss (1.79%) compared to the control group and O_3 (24 h) treatment, which recorded losses of 2.58% and 2.60%, respectively. These findings align with those reported by Bambalele et al. [15], who observed that a 0.25 mg/L O_3 treatment preserved mango fruit firmness for up to 14 days. Similarly, Zhang et al. [41] found that O_3 (1 mg/L for 5h) treatment effectively maintained the firmness of cantaloupe melons stored at 4°C for 30 days. The preservation of firmness in these fruits is likely due to the capacity of O_3 to inhibit the activity of cell wall-degrading enzymes, such as

polygalacturonase and β -galactosidase [15].

Fruit firmness is fundamentally dependent on the structural integrity of the cell wall, which consists of polysaccharides such as pectin, cellulose, hemicellulose, and lignin, all of which contribute to its strength [42]. The enzymatic degradation and solubilization of cell wall polysaccharides result in modifications of the cell wall's structure and composition, leading to textural changes that manifest as fruit softening [42]. Loss of firmness in fruit may be attributed to alterations in the structural components of the cell wall, turgor pressure variations, and changes in intercellular adhesion [43]. Additionally, gradual cellular degradation during storage due to senescence further exacerbates the loss of fruit firmness [44].

3.5. Catalase

Catalase (EC 1.11.1.6) protects fruit against fungal infections by decomposing H_2O_2 into water and oxygen, thereby mitigating oxidative stress [45]. In this study, O_3 treatment significantly ($p < 0.001$) influenced CAT activity of the treated lemons (Fig. 5). However, the interaction between storage conditions and treatment did not have a statistically significant ($p > 0.05$) effect on CAT activity. Fruit treated with O_3 (24 h) showed the highest increase in CAT activity (47%) from Day 0 to Day 35, compared to the control and O_3 (48 h) treatment, which was 44% and 17%, respectively. There is limited literature regarding the effects of O_3 on CAT activity in citrus fruits, making this study one of the few to investigate this relationship. The findings align with those of Zhang et al. [46], who reported similar results in strawberries. The increase in CAT activity may be attributed to ozone-induced stimulation of antioxidant enzymes, which enhances the fruit's defence against oxidative damage [38]. Elevated CAT activity facilitates the scavenging of superoxide anions and H_2O_2 during storage [46]. Essentially, the potent oxidizing effect of O_3 triggers the upregulation of CAT, which aids in neutralizing ROS and preserving fruit quality during storage.

In the O_3 (48 h) treatment, CAT activity showed a rising trend from Day 0 to Day 28. However, a sudden decline in CAT activity was observed during ambient storage. This decline may be linked to temperature fluctuations that affect the fruit's response to free radicals. Although O_3 initially enhances CAT activity, prolonged exposure to O_3 can lead to decreased activity [47]. Additionally, the decline in CAT activity may result from oxidative modifications to the enzyme due to excessive oxidative stress induced by O_3 [48].

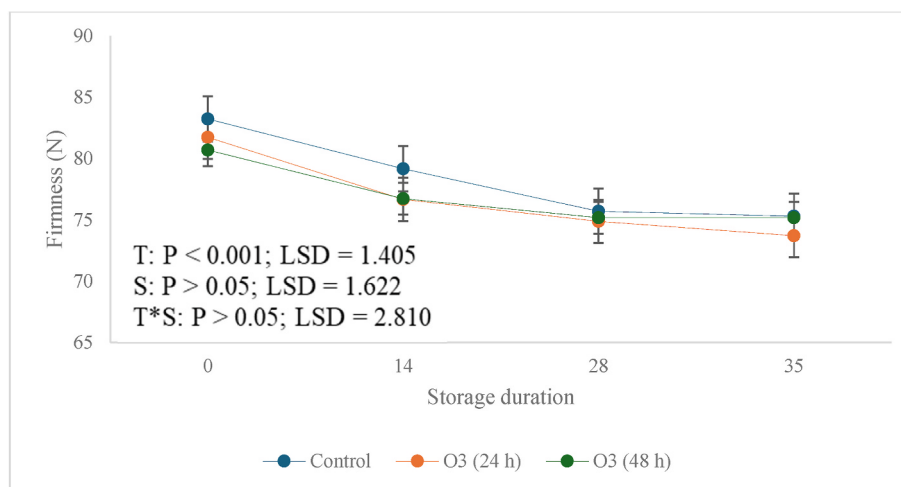


Fig. 4. Effect of gaseous ozone on the firmness loss of 'Eureka' lemons during cold sterilization at 0°C for 28 days and shelf-life at ambient temperature for seven days ($\pm\text{SE}$, $n = 5$).

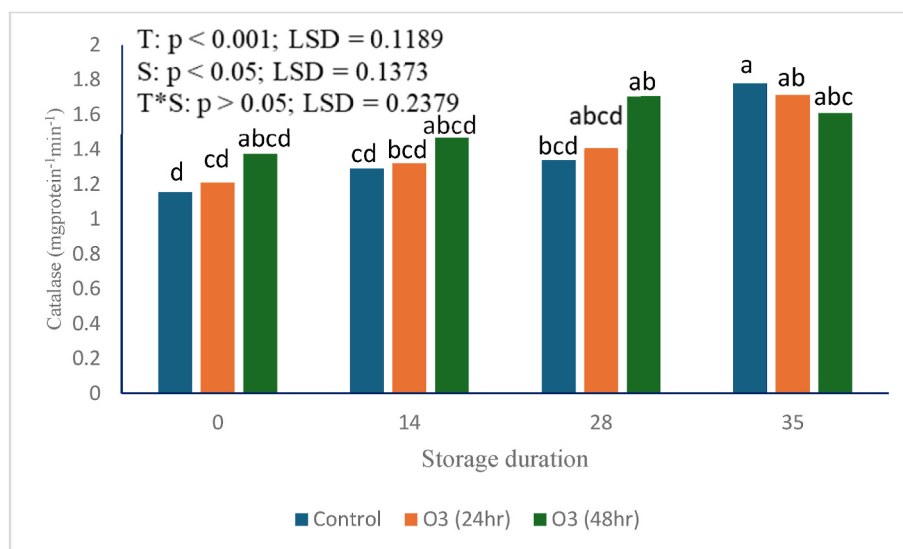


Fig. 5. Effect of gaseous ozone on catalase concentration of 'Eureka' lemon peel during cold sterilization at 0 °C for 28 days and shelf-life at ambient temperature for seven days ($\pm$ SE, $n = 3$).

3.6. Peroxidase

The interaction between treatment and storage time did not significantly affect POD activity ($p > 0.05$). However, they both significantly influenced POD activity individually in 'Eureka' lemons ($p < 0.001$). The current findings demonstrate a consistent increase in POD activity across all treatments (Control, O₃(24 h), and O₃(48 h) (Fig. 6). Remarkably, the longer ozone exposure period resulted in higher POD activity. These results are consistent with those reported by Zhu et al. [49], who found that O₃ (2.5 μ g/L for 24 h) enhanced the POD activity in 'Satsuma' mandarins over a 30-days storage period. Similarly, Xu et al. [50] reported an increased POD activity in coriander exposed to 0.68 mg/L of O₃ for 10 min. Peroxidase activity is known to change during fruit storage and ripening, and it is strongly linked to enzymatic browning, cellular damage, and quality deterioration. Thus, the elevated POD activity in ozone-treated fruit could be a response to cellular damage, rind breakdown, and decay incidence caused by the ozone and low temperatures.

3.7. Lipxygenase

The interaction between treatment and storage duration did not yield a statistically significant effect ($p > 0.05$) on the LOX levels in 'Eureka' lemons. However, both treatment type and storage duration had a significant impact ($p < 0.001$) on the concentration of LOX in the lemon peel. The control and the O₃ (24 h) treatments exhibited a similar upward trend in LOX enzyme activity (Fig. 7). These results are consistent with Modesti et al. [51], who indicated that ozonated wine grapes (20 g h⁻¹ over 3 days) experienced a more pronounced increase in LOX activity compared to untreated fruit and those treated with methyl jasmonate at concentrations of 10 μ M and 100 μ M. The increased LOX activity is often a marker of membrane lipid peroxidation and senescence. For almost all the treatments, LOX activity showed an upward trend, which corresponded with decay incidence. Notably, from Day 0 to Day 28, the LOX activity was higher in O₃ (48 h) compared to O₃ (24 h). The heightened LOX concentration observed in ozonated fruit may therefore reflect enzyme activation by O₃ [14]. As earlier

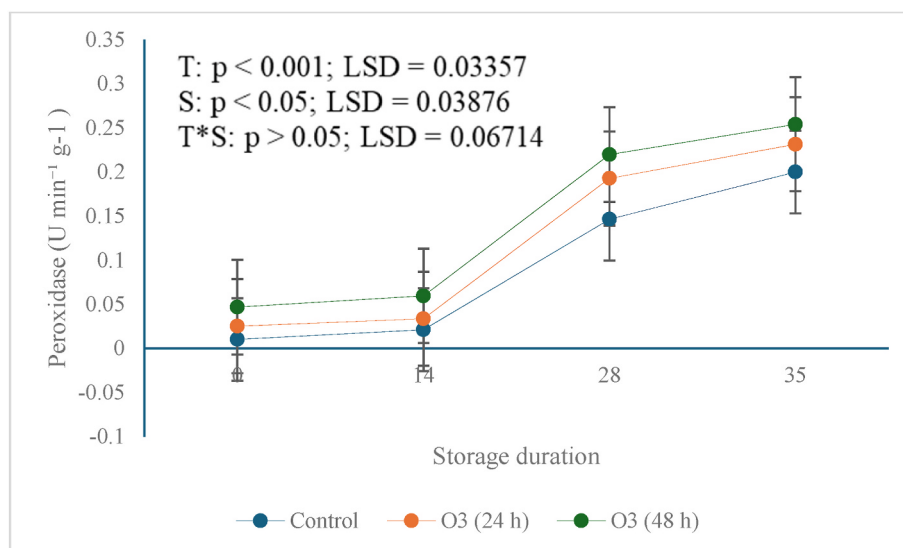


Fig. 6. Effect of gaseous ozone on peroxidase of 'Eureka' lemon peel during cold sterilization at 0 °C for 28 days and shelf-life at ambient temperature for seven days ($\pm$ SE, $n = 3$).

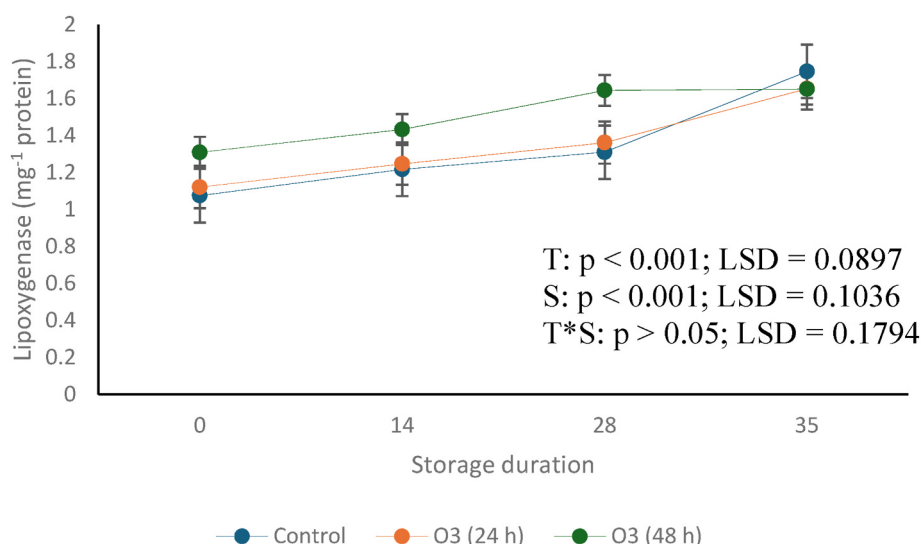


Fig. 7. Effect of gaseous ozone on lipoxigenase of 'Eureka' lemon peel during cold sterilization at 0 °C for twenty-eight days and shelf-life at ambient temperature for seven days ($\pm$ SE, $n = 3$).

mentioned, gaseous ozone can induce oxidative stress in fruit tissues, potentially activating LOX and compromising rind integrity. LOX damages cells by oxidizing polyunsaturated fatty acids into hydroperoxides, triggering lipid peroxidation chains that rupture membranes, leak solutes, and collapse fruit structure [6,10,27].

Although O₃(48 h) also resulted in increased LOX activity, the levels declined between Days 28 and 35. As a stress-inducing agent, prolonged O₃ exposure may disrupt enzymatic pathways, reducing LOX activity [52]. The elevated ROS levels are associated with the activation of antioxidant pathways, which may downregulate LOX activity [53,54].

3.8. Glutathione reductase

Glutathione reductase (GR) is an important enzymatic antioxidant in lemon fruit as it maintains redox balance and minimizes oxidative stress during cold storage and postharvest handling. In this study, the GR levels were significantly influenced by the treatment ($p < 0.001$), while neither storage duration nor their interaction had a significant effect ($p > 0.05$) on GR enzyme activity. All treatment groups (Control, O₃ 24 h

and 48 h) exhibited a consistent upward trend in GR activity throughout the storage period (Fig. 8). Except during the shelf-life, both ozone treatments constantly had higher GR compared to the control treatment, with O₃ (48 h) recording the highest concentration. These findings align with Piechowiak et al. [47], who reported increased GR activity in raspberry, 'Highbush' blueberry, strawberry, blackberry, and blackcurrant (15 mg/L for 30 min). The increase was attributed to the enhanced mitochondrial function and ATP production, which are essential for GR synthesis. The high GR levels in ozone-treated fruit could be attributed to the fact that GR is primarily triggered by oxidative stress, which prompts the fruit to produce antioxidants as a defence mechanism.

The observed decline in GR activity on Day 35 for O₃(48 h) treated fruit may be ascribed to the combined effects of prolonged O₃ exposure and extreme temperature during cold sterilization. While initial ozonation may enhance enzyme activities, extended storage often results in diminished enzymatic function due to accumulated oxidative stress and nutrient depletion [46].

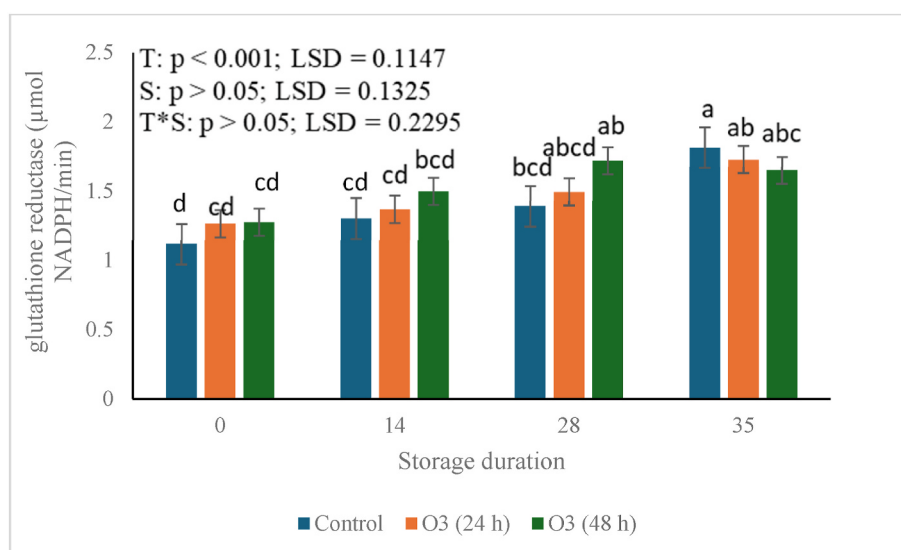


Fig. 8. Effect of gaseous ozone on glutathione reductase of 'Eureka' lemon peel during cold sterilization at 0 °C for 28 days and shelf-life at ambient temperature for seven days ($\pm$ SE, $n = 3$).

3.9. Lipid peroxidation

Lipid peroxidation is a significant factor contributing to the degradation of food quality during storage and processing. This process involves the oxidation of unsaturated fatty acids within cellular membranes, resulting in considerable damage to fruit tissues [55]. Lipid peroxidation leads to the generation of various oxidative byproducts, including H_2O_2 , hydroxyl radicals, and reactive aldehydes, which can adversely affect proteins and contribute to overall deterioration [56]. Statistically, through all the storage days (D_0 to D_{35}) and treatments (Control, O_3 (24 h) and O_3 (48 h), lipid peroxidation was not significantly ($p > 0.05$) affected (data not shown). Excessive exposure of fresh produce to O_3 may induce lipid peroxidation by triggering ROS formation, which can damage cellular lipids. For instance, in their study on 'Clementina' mandarin, Iglesias et al. [57] found that O_3 increased lipid peroxidation at the end of the 12-month storage to $98.3 \text{ nmol MDA g}^{-1} \text{ FW}$. Although O_3 may stimulate the fruit's defence system against stress [58], prolonged treatment may be detrimental to membrane integrity. While the ozone treatments had an insignificant impact on lipid peroxidation in this study, the increased LOX activity is a cause for concern.

4. Conclusion

This study demonstrated that O_3 did not significantly affect mass loss, colour, and lipid peroxidation while slightly retaining firmness in 'Eureka' lemon fruits. However, decay incidence in O_3 -treated fruits was notably higher than in the control treatment. This could be linked to the rind breakdown caused by cold sterilization plus ozone-induced oxidative stress. Although ozone treatment enhanced enzymatic antioxidants (glutathione reductase and catalase), the elevated levels of peroxidase and lipoxygenase are an indication that the use of O_3 during cold sterilization could be detrimental for membrane integrity and overall quality of 'Eureka' lemons. Further research is warranted to optimize O_3 exposure duration in order to minimize the accumulation of LOX and POD, suppress rind breakdown, and fruit decay.

CRediT authorship contribution statement

Hlumelo Mhlwatika: Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Nonjabulo L. Bambalele:** Writing – review & editing, Validation, Methodology, Data curation. **Olaoluwa O. Olarewaju:** Writing – review & editing, Validation, Data curation. **Samson Z. Tesfay:** Writing – review & editing, Supervision, Methodology, Investigation, Formal analysis, Conceptualization. **Lembe S. Magwaza:** Writing – review & editing, Validation, Investigation, Conceptualization. **Asanda Mditshwa:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Investigation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This research was financially supported by the National Research Foundation Support for Y-rated Researchers Grant (CSRP: 2205046155). The authors are grateful to Thokozani Nkosi for technical assistance in the laboratory.

Data availability

Data will be made available on request.

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The effect of short-term gaseous ozone treatment on physical attributes and senescence-related enzymes of 'Eureka' lemons during cold sterilization

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2026-05

Elsevier

<https://doi.org/10.1016/j.jafr.2026.102788>

Mhlwatika H, Bambalele NL, Olarewaju OO, et al., (2026) The effect of short-term gaseous ozone treatment on physical attributes and senescence-related enzymes of 'Eureka' lemons during cold sterilization. Journal of Agriculture and Food Research, Volume 27, May 2026, Article number 102788