Effect of Gaseous Ozone and Heat Treatment on Quality and Shelf Life of Fresh Strawberries during Cold Storage

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ABSTRACT
Ozone is a powerful oxidant agent which is used in foods preservation on account of its ability of killing microorganisms. In the present study, the combined effects of ozone gas treatment and heat treatment on the physicochemical and sensory characteristics and shelf-life of strawberries kept under refrigeration were investigated. Strawberries were heat treated, ozonated at concentrations of 0.5, 1.0, and 1.5 ppm for 40 min and ozonated at the above-mentioned conditions in combination with heat treatment. Ozone and heat treatment affected significantly (p < .05) weight loss percentage and titratable acidity. Total soluble solids (TSS) content was affected significantly by heat treatment, ozone treatment at concentrations of 0.5 and 1.0 ppm and by the ozone treatments at all concentrations plus heat treatment. Color parameters L*, a*, b*, and pH were affected significantly by ozonation at concentration of 1.5 ppm plus heat treatment. Firmness, pH, and color parameter L* were affected significantly by ozonation at 1.0 ppm. Heat treatment and ozonation at 1.0 ppm plus heat treatment also affected firmness. In conclusion, strawberries treated with ozone at a concentration of 0.5 ppm and at 0.5 ppm plus heat treatment recorded a higher score in sensory analysis and slightly longer storage time.

KEYWORDS
Ozone; strawberries; cold storage; heat treatment

Introduction
Strawberry (Fragaria ananassa) is a fruit that has some advantages and some disadvantages. The advantages refer to the high nutritional value (Kerch et al., 2011; Li et al., 2020) and the high antioxidant properties (Cordenunsi et al., 2002; Lu et al., 2018). The disadvantages refer to the short postharvest life because of the easy growth of harmful microorganisms during storage (Kim et al., 2010; Williams and Kim, 1995; Yan et al., 2019) and the high softening rate (Jing et al., 2008). The combination of using pesticides and storage under refrigerated condition is a solution to avoid postharvest decay of strawberry (Bao and Liu, 2004; Li et al., 2017; Qian et al., 2006; Washington et al., 1992). There are some problems with the usage of fungicides in fruits such as the negative effect on the environment and human health and the development fungicide resistance by fungi (Cao et al., 2010; Gabler et al., 2010; Xu et al., 2019). On account of these problems, there is a need to seek alternative non-damaging treatments to maintain strawberry quality. Three of the most effective treatments are those with gaseous ozone, Blanching and gamma irradiation (Majeed et al., 2014; Nadas et al., 2003; Perez et al., 1999; Vicente et al., 2002).

Heat treatment has been studied in postharvest life of strawberry. Barkal-Golan and Phillips (1991) and Shao et al. (2007) reported that heat treatment was effective to kill or inactivate plant pathogen microorganisms, control insect’s attacks and maintain the quality of fruit during cold storage. Garcia et al. (1995) reported a significant reduction of postharvest losses of strawberry. The success of a heat
Treatment is due to a combination of killing the most harmful and pathogenic microorganisms and maintaining the quality characteristics (Luo et al., 2009). A new hot water treatment technology involves immersion in hot water (55–65°C) for a short time (10–30s). This technology has proven to be effective in controlling the decay and removal of pesticides without any negative change to the quality characteristics of the fruit (Garcia et al., 1995; Couey and Pollstad, 1966; Porat et al., 2000a, 2000b; Wszelaki and Mitcham, 2002; Vicente et al., 2003; Fallik, 2004; Jing et al., 2008). This technology was successfully used to preserve the freshness of mango (Prusky et al., 1999).

Ozone is a triatomic oxygen molecule that has a high oxidative capacity and a potent antimicrobial activity against numerous species of microorganisms (Khadre et al., 2001). Ozone can be applied in gaseous form or in aqueous solution. On a commercial scale, ozone should be produced on site and in the USA it is designated GRAS (Generally recognized as Safe) for food contact applications (U.S. Food and Drug Administration, 2001). Ozone doesn’t leave residues on treated fruits and its germicidal effect is based on its oxidative effect on cell membranes of microorganisms (Cataldo, 2003). Ozone is used as a water disinfectant in many parts of the world.

In a previous study, the effect of gamma-irradiation on sensory characteristics, physicochemical parameters, and shelf life of strawberries during cold storage was studied (Panou et al., 2019). The aim of the present study was the effect of combined gaseous ozone and heat treatment on a number of basic physicochemical and sensory characteristics and the shelf life of strawberries stored under refrigerated conditions.

Materials and Methods

Materials

Fresh strawberries (cv. Carbarosa, cultivated in Corinth- Greece) were obtained from the local vegetable market of Ioannina-Greece and were immediately transported to the laboratory.

Gaseous ozone was supplied by a C-Lasky series, model L010 ozone generator (Air tree company, Taiwan) equipped with an OS-4 Eco-Sensor (Santa Fe, New Mexico, USA).

Experimental Design

Strawberries were thoroughly washed with tap water and were wiped with absorbent paper. The strawberries were then subjected to the following treatments:

1st treatment: control (untreated) sample [ctl]
2nd treatment: immersion in hot water at a temperature of 55°C for 15 s [HT]
3rd treatment: application of gaseous ozone at a concentration 0.5 ppm for 40 minutes [0.5 ppm]
4th treatment: application of gaseous ozone at a concentration 1.0 ppm for 40 minutes [1.0 ppm]
5th treatment: application of gaseous ozone at a concentration 1.5 ppm for 40 minutes [1.5 ppm]
6th treatment: application of gaseous ozone at a concentration 0.5 ppm for 40 minutes plus immersion in hot water at a temperature of 55°C for 15 s [0.5 ppm + HT]
7th treatment: application of gaseous ozone at a concentration 1.0 ppm for 40 minutes plus immersion in hot water at a temperature of 55°C for 15 s [1.0 ppm + HT]
8th treatment: application of gaseous ozone at a concentration 1.5 ppm for 40 minutes plus immersion in hot water at a temperature of 55°C for 15 s [1.5 ppm + HT]

The temperature of 55°C was used, as it was found that the treatment at temperatures higher than 55°C caused undesirable changes in the organoleptic characteristics of the fruit and especially in their texture (softening).

Finally, strawberries from all treatments were stored under refrigerated conditions (1°C and relative humidity 90%, in a refrigerator model FR280, ELVEM – Construction, Trading and Apparatus Service, Spata-Attica, Greece) for 15 days. Weight loss percentage, texture analysis, total soluble solids
content, color values (L*, a* and b*), titratable acidity, pH, and sensory analysis (appearance, taste, and odor) were monitored every 5 days during cold storage.

**Weight Loss Percentage**

In all treatments, 300 g strawberries were weighted in a plastic container. Their weight was recorded every 4 days. Weight loss percentage calculation was done according to the following equation:

\[
\text{weightloss(\%)} = \frac{\text{weight}(i) - \text{weight}(t)}{\text{weight}(i)} \times 100\% \quad (1)
\]

where weight (i) = the initial weight of berries, weight (t) = the weight of berries at time t.

The results were expressed as the mean of three replicates.

**Texture Analysis**

Ten strawberries were randomly picked and used for texture analysis. An Instron 4411 dynamometer (Instron Ltd, Buckinghamshire, UK) was used for the calculation of the force needed to puncture their surface. Instron 4411 dynamometer consist of a cylindrical probe (diameter 4 mm) moved with a velocity of 10 mm/min. Both sides were measured in each strawberry. The results were expressed as the mean of 20 measurements.

**Total Soluble Solids Content**

Ten strawberries were randomly picked and squeezed by hand pressing. Their juice was placed in the prism of a digital refractometer (JENA, Germany) previously calibrated with distilled water. The results were expressed in Brix degrees.

**Color Parameters, Titratable Acidity and pH Measurements**

**Color Parameters (L*, A* and B*)**

150 g strawberries were crushed and filtered. The filtrate was transferred into a cylindrical (diameter 11.3 cm, height 2 cm) optical cell. Color parameter measurements were performed using a Hunter Lab model DP-9000 optical sensor colorimeter (Hunter Associates Laboratory, Reston, VA, USA). A 45 mm viewing aperture was used to read the reflectance values. The instrument was calibrated using a white and black tile. For each treatment, two (2) replications were performed. Five (5) readings were made for each replicate. The results were expressed as the average of 10 measurements.

**Titratable Acidity and pH Measurements**

5 g of crushed strawberries were mixed with 50 ml of distilled water. The mixture was homogenized and filtered through a paper filter. The filtrate was used for pH measurements using a pH-meter (Microprocessor pH Meter, HANNA Instruments, Romania) equipped with a glass electrode. All samples were measured in duplicate. Moreover, 5 ml of the filtrate were transferred into a 250 ml Erlenmeyer flask, diluted with 45 ml of distilled water and titrated with a standard 0.1 M sodium hydroxide solution using phenolphthalein as indicator. All samples were analyzed in duplicate. The results were the average of the two measurements and were expressed as g of citric acid per 100 g of strawberry.

**Descriptive Sensory Analysis**

A panel of seven panelists was used for the sensory characteristics estimation. This panel was trained in discrimination of small differences in sensory characteristics among the treatments. The estimated sensory characteristics were general appearance, taste, and odor. After examining the taste and odor,
the panelists were asked to record if there was any particular feature in the tested samples. The panelists cleaned their palate by eating bread and sipping water. A nine-point scale was used for the estimation of the above sensory characteristics. This scale was as follows:

9 = excellent, 7 = very good, 5 = good, 3 = moderate and 1 = not acceptable

The limit of acceptability was set to 5.

Statistical Analysis
Results were tested for normality of distribution and homogenous variance and were subjected to one-way analysis of variance (ANOVA) using software SPSS 16 for windows. The level of significance was determined using the least significance difference calculated at the $P < .05$ level. F, df, and $p$ values of the calculated physicochemical parameters for all treatments are given in Table 1.

Results and Discussion

Weight Loss Percentage

The results for the weight loss percentage are shown in Figure 1. An increase in the weight loss percentage was observed in all treatments due to water loss during respiration. Water loss rate depends on respiration rate, fluctuations of temperature and relative humidity levels. Weight loss rates were significantly ($p < .05$) increased in all treatments compared to the control sample during cold storage.

All stored commodities suffer from weight loss due to loss of water during storage under refrigerated conditions. This is one of the main disadvantages of stored agricultural products. The loss of water occurs through the pores of the skin of the fruit and is due to changes in the storage temperature and relative humidity of the storage room (Aghdam et al., 2018). In our results, all processed strawberries had a higher weight loss percentage compared to untreated ones, throughout the cold storage period. Previous studies have also shown a significant increase in the percentage of weight loss in fruits ozonated at high concentrations compared to untreated fruits due to the damage of the epidermal tissue and the appearance of injured areas (Palou et al., 2002).

Firmness

The results of the texture analysis are shown in Figure 2. A reduction in firmness was observed in all treatments during cold storage. This reduction can be attributed to the hydrolysis of pectin by polygalacturonase during ripening. Strawberries treated with 1.0 ppm ozone and 1.0 ppm ozone plus heat treatment showed statistically significantly higher ($p < .05$) firmness compared to untreated strawberries during cold storage.

Firmness is an important feature of a fruit as many consumers use it as a criterion for evaluating its quality (Mou et al., 2015). Fruits firmness changes during ripening and storage due to the degradation of protopectin to pectin by the action of polygalacturonase, the hydrolysis of methyl esters by the action of pectin methyl esterase and the degradation of cellulose and hemicelluloses (Cheng et al., 2009; Duan et al., 2008; Luo, 2006). The activity of these two enzymes can be adversely affected by ozone-induced oxidation. Previous studies have reported a correlation between a decrease in the firmness of grapes and pears and an increase in water soluble content and a decrease in cellulose and hemicellulloses (Chen et al., 2017; Deng et al., 2005). Dotto et al. (2011) found lower firmness in strawberries treated in an oven at 45°C for 3 hours after 1 day of storage. Similar results were found by Vicente et al. (2005). Strawberry’s firmness treated at 55°C, 60°C, and 65°C was higher compared to untreated strawberries during cold storage (Jing et al., 2008). Similar results also found Garcia et al. (1995). Lara et al. (2006) reported that thermal treatments maintain the firmness of strawberries. Santos Pedro et al. (2006) treated strawberries at 60°C and found that these had higher firmness than untreated strawberries. During thermal treatment polygalacturonase and b-galactosidase are inactivated, while the activity of pectin methyl esterase is increased resulting in a slowdown in the rate of pectin degradation (Pan et al., 2004; Vicente et al., 2005). The effect of heat...
Table 1. F, df and p values of the physicochemical parameters measured for all treatments.

| Parameter | A. Weight loss | B. Firmness | C. Total soluble solids (TSS) | D. Titratable acidity | E. pH | F. Color parameter L* | G. Color parameter a* | H. Color parameter b* |
|-----------|---------------|-------------|-------------------------------|-----------------------|------|----------------------|----------------------|----------------------|
|           | F             | df          | p                             |                        |      |                      |                      |                      |
| Ctl vs. ctrl + ht | -10.757 | 14 | 1.69x10^{-14} < 0.05 |                        |      |                      |                      |                      |
| Ctl vs. 0.5 ppm | -9.958 | 14 | 2.31x10^{-13} < 0.05 |                        |      |                      |                      |                      |
| Ctl vs. 1.0 ppm | -10.410 | 14 | 5.21x10^{-14} < 0.05 |                        |      |                      |                      |                      |
| Ctl vs. 1.5 ppm | -10.996 | 14 | 7.84x10^{-13} < 0.05 |                        |      |                      |                      |                      |
| Ctl vs. 0.5 ppm + ht | -11.185 | 14 | 4.29x10^{-15} < 0.05 |                        |      |                      |                      |                      |
| Ctl vs. 1.0 ppm + ht | -12.84 | 14 | 2.69x10^{-17} < 0.05 |                        |      |                      |                      |                      |
| Ctl − 1.5 ppm + ht | -11.086 | 14 | 5.87x10^{-15} < 0.05 |                        |      |                      |                      |                      |
|           | F             | df          | p                             |                        |      |                      |                      |                      |
| Ctl vs. ctrl + ht | -8.457 | 99 | 3.85x10^{-11} < 0.05 |                        |      |                      |                      |                      |
| Ctl vs. 0.5 ppm | 3.381 | 99 | 0.14 > 0.05 |                        |      |                      |                      |                      |
| Ctl vs. 1.0 ppm | -4.292 | 99 | 8.3x10^{-6} < 0.05 |                        |      |                      |                      |                      |
| Ctl vs. 1.5 ppm | -5.927 | 99 | 0.303 > 0.05 |                        |      |                      |                      |                      |
| Ctl vs. 0.5 ppm + ht | -0.559 | 99 | 0.579 > 0.05 |                        |      |                      |                      |                      |
| Ctl vs. 1.0 ppm + ht | -10.216 | 99 | 9.85x10^{-14} < 0.05 |                        |      |                      |                      |                      |
| Ctl vs. 1.5 ppm + ht | 2.532 | 99 | 0.15 > 0.05 |                        |      |                      |                      |                      |
|           | F             | df          | p                             |                        |      |                      |                      |                      |
| Ctl vs. ctrl + ht | -2.234 | 49 | 0.03 < 0.05 |                        |      |                      |                      |                      |
| Ctl vs. 0.5 ppm | 5.724 | 49 | 6.21x10^{-7} < 0.05 |                        |      |                      |                      |                      |
| Ctl vs. 1.0 ppm | 7.434 | 49 | 1.41x10^{-9} < 0.05 |                        |      |                      |                      |                      |
| Ctl vs. 1.5 ppm | -3.174 | 49 | 0.151 > 0.05 |                        |      |                      |                      |                      |
| Ctl vs. 0.5 ppm + ht | 8.127 | 49 | 1.22x10^{-10} < 0.05 |                        |      |                      |                      |                      |
| Ctl vs. 1.0 ppm + ht | 10.441 | 49 | 4.7x10^{-14} < 0.05 |                        |      |                      |                      |                      |
| Ctl vs. 1.5 ppm + ht | 3.607 | 49 | 0.001 < 0.05 |                        |      |                      |                      |                      |
|           | F             | df          | p                             |                        |      |                      |                      |                      |
| Ctl vs. ctrl + ht | 10.822 | 9 | 1.37x10^{-14} < 0.05 |                        |      |                      |                      |                      |
| Ctl vs. 0.5 ppm | 9.473 | 9 | 1.17x10^{-12} < 0.05 |                        |      |                      |                      |                      |
| Ctl vs. 1.0 ppm | 13.429 | 9 | 4.82x10^{-18} < 0.05 |                        |      |                      |                      |                      |
| Ctl vs. 1.5 ppm | 9.28 | 9 | 2.26x10^{-12} < 0.05 |                        |      |                      |                      |                      |
| Ctl vs. 0.5 ppm + ht | 12.033 | 9 | 3.05x10^{-16} < 0.05 |                        |      |                      |                      |                      |
| Ctl vs. 1.0 ppm + ht | 12.133 | 9 | 2.24x10^{-16} < 0.05 |                        |      |                      |                      |                      |
| Ctl vs. 1.5 ppm + ht | 10.152 | 9 | 1.21x10^{-13} < 0.05 |                        |      |                      |                      |                      |
|           | F             | df          | p                             |                        |      |                      |                      |                      |
| Ctl vs. ctrl + ht | -3.296 | 9 | 0.17 > 0.05 |                        |      |                      |                      |                      |
| Ctl vs. 0.5 ppm | -4.618 | 9 | 0.28 > 0.05 |                        |      |                      |                      |                      |
| Ctl vs. 1.0 ppm | 0.469 | 9 | 0.01 < 0.05 |                        |      |                      |                      |                      |
| Ctl vs. 1.5 ppm | -2.672 | 9 | 0.17 > 0.05 |                        |      |                      |                      |                      |
| Ctl vs. 0.5 ppm + ht | -4.003 | 9 | 0.212 > 0.05 |                        |      |                      |                      |                      |
| Ctl vs. 1.0 ppm + ht | -2.513 | 9 | 0.015 < 0.05 |                        |      |                      |                      |                      |
| Ctl vs. 1.5 ppm + ht | 1.174 | 9 | 0.246 > 0.05 |                        |      |                      |                      |                      |
|           | F             | df          | p                             |                        |      |                      |                      |                      |
| Ctl vs. ctrl + ht | 4.139 | 49 | 0.137 > 0.05 |                        |      |                      |                      |                      |
| Ctl vs. 0.5 ppm | 1.021 | 49 | 0.312 > 0.05 |                        |      |                      |                      |                      |
| Ctl vs. 1.0 ppm | -3.817 | 49 | 379x10^{-6} < 0.05 |                        |      |                      |                      |                      |
| Ctl vs. 1.5 ppm | 0.508 | 49 | 0.614 > 0.05 |                        |      |                      |                      |                      |
| Ctl vs. 0.5 ppm + ht | 0.575 | 49 | 0.568 > 0.05 |                        |      |                      |                      |                      |
| Ctl vs. 1.0 ppm + ht | 1.474 | 49 | 0.147 > 0.05 |                        |      |                      |                      |                      |
| Ctl vs. 1.5 ppm + ht | -4.481 | 49 | 45x10^{-6} < 0.05 |                        |      |                      |                      |                      |
Table 1. (Continued).

|               | F     | df | p          |
|---------------|-------|----|------------|
| Ctl vs. ctl + ht | 6.021 | 49 | 2.17x10⁻⁷ < 0.05 |
| Ctl vs. 0.5 ppm | −2.062 | 49 | 0.045 < 0.05       |
| Ctl vs. 1.0 ppm | −3.127 | 49 | 0.003 < 0.05       |
| Ctl vs. 1.5 ppm | 8.133  | 49 | 1.19x10⁻¹⁰ < 0.05   |
| Ctl vs. 0.5 ppm + ht | 3.09  | 49 | 0.003 < 0.05       |
| Ctl vs. 1.0 ppm + ht | 3.09  | 49 | 0.003 < 0.05       |
| Ctl vs. 1.5 ppm + ht | 2.735 | 49 | 0.211 > 0.05       |

Figure 1. Effect of ozone and treatment on weight loss percentage of strawberries during cold storage.

Figure 2. Effect of ozone and heat treatment on firmness of strawberries during cold storage.
treatment on these enzymes related to pectin metabolism explains the lower solubility of pectin in heat-treated fruits (Vicente et al., 2005). Ozone also breaks down ethylene and slows down the rate of pectin degradation during ripening.

**Total Soluble Solids**

The results for the total soluble solids content are shown in Figure 3. Total soluble solids (TSS) include carbohydrates (monosaccharides and disaccharides), proteins, free amino acids, and non-organic matter. A decrease in TSS content was observed in all treatments during cold storage, due to the consumption of carbohydrate during fruit respiration. Heat-treated, ozone-treated strawberries at concentrations of 0.5 ppm and 1.0 ppm and ozone-treated at all concentrations plus heat treatment showed a statistically significant lower (p < .05) TSS content compared to the unprocessed strawberries during cold storage.

Total soluble solids provide much information on the content of sugars, organic acids and inorganic compounds and their fluctuations during storage. Other ingredients at lower concentrations are proteins, free amino acids, and lipids. During storage, a decrease in TSS content was observed in all treatments due to the consumption of sugars during respiration. An increase was observed during the last days of sampling probably due to the TSS condensation in the liquid phase. Ozonation at 0.5 and 1.0 ppm resulted in a higher TSS content compared to the control sample during cold storage, while the same results were also observed with the combined ozonation and heating treatment. Ozonation may have caused oxidative shock to the metabolism of sugars, resulting in a shift in their metabolic pathway which becomes slower. Wszelaki and Mitcham (2003) treated strawberries at 63°C for 12 s and found lower TSS content compared to untreated ones after 5 days of storage. However, after 14 days of storage, the heat-treated strawberries had a significantly higher TSS content compared to the untreated ones.

**Titratable Acidity**

The results for the titratable acidity are shown in Figure 4. A decrease in titratable acidity was observed in all treatments during cold storage due to the disruption of organic acids occurring during strawberry ripening. The rate of decrease in titratable acidity increased significantly (p < .05) in all treatments compared to untreated strawberries during cold storage.

Titratable acidity is an indicator of total organic acids content of a fruit. During ripening, titratable acidity decreases due to the high participation of organic acids in the reactions of citric acid cycle and

![Figure 3. Effect of ozone and heat treatment on TSS content of strawberries during cold storage.](image)
other decarboxylation reactions. Any applied ozone treatment and combined ozone treatment with heating increase the rate of reduction of titratable acidity compared to the control sample during cold storage. Wszelaki and Mitcham (2003), reported also higher titratable acidity in untreated strawberries as compared to strawberries treated at 63°C for 12 s throughout the cold storage.

**pH Measurements**

The results of pH measurements are shown in Figure 5. In general, an increase in pH values was observed in all treatments during cold storage. Ozone treatment at a concentration of 1.0 ppm and at a concentration of 1.5 ppm plus heat treatment affected significantly ($p < .05$) the pH values compared to untreated strawberries during cold storage.

None of the treatments applied affected the pH during cold storage compared to control sample, except for ozonation at 1.0 ppm and 1.5 ppm plus heat treatment. According to Alexander et al. (2012), no significant changes in pH of strawberries treated with aqueous ozone were found. Also, the addition
of other oxidizing agents such as chlorine dioxide to lettuce and cabbage did not cause significant changes in pH (Gomez-Lopez et al., 2008).

**Color Parameters (L*, A* and B*)**

The results of the color parameter measurements are shown in Table 2–4. Food color is one of the major characteristics of the food quality, as it is directly related to the appearance of a food, which is the first consumer-rated feature. L*, a*, and b* parameters indicated the gradations of lightness and darkness, redness and greenness, and yellowness and blueness of a food, respectively.

An increase in the L* parameter was observed in all treatments up to the 8th day of sample storage. After the 8th day of storage, the L* parameter decreased due to the oxidation of phenolic substrates. Heat-treated strawberries showed a slight increase in the L* parameter during cold storage. This could be attributed to the inactivation of the enzymes involved in the browning reactions. Ozone-treated strawberries at a concentration of 1.0 ppm and at a concentration of 1.5 ppm plus heat treatment, showed statistically significantly higher values of L* (p < .05) compared to control samples during cold storage. L* parameter of a plant product can be affected by surface injuries, the enzymatic action of polyphenoloxidase on phenolic substrates and the structure of the cell wall biopolymers (Luo et al., 2008).

| Table 2. Effect of ozone and heat treatment on the color parameter L* of strawberries during cold storage. |
|---------------------------------------------------------------|
| **Days of storage** | **Control** | **Control + HT** | **0.5 ppm ozone** | **1.0 ppm ozone** | **1.5 ppm ozone** | **0.5 ppm ozone + HT** | **1.0 ppm ozone + HT** | **1.5 ppm ozone + HT** |
|---------------------|-------------|-----------------|------------------|------------------|------------------|-----------------------|-----------------------|-----------------------|
| 0                   | 26.64 ±0.35 | 26.64 ±0.35     | 26.64 ±0.35      | 26.64 ±0.35      | 26.64 ±0.35      | 26.64 ±0.35           | 26.64 ±0.35           | 26.64 ±0.35           |
| 4                   | 27.11 ±0.41 | 26.53 ±0.43     | 27.56 ±0.36      | 29.52 ±0.52      | 27.89 ±0.79      | 28.63 ±0.53           | 27.63 ±0.43           | 29.29 ±0.39           |
| 8                   | 28.09 ±0.49 | 26.30 ±0.40     | 27.80 ±0.59      | 28.68 ±0.58      | 27.60 ±0.59      | 27.89 ±0.65           | 27.23 ±0.43           | 28.70 ±0.49           |
| 12                  | 29.05 ±0.39 | 26.36 ±0.41     | 27.69 ±0.75      | 27.83 ±0.65      | 27.66 ±0.55      | 27.16 ±0.56           | 27.00 ±0.61           | 28.24 ±0.54           |
| 16                  | 26.20 ±0.80 | 27.32 ±0.52     | 26.84 ±0.54      | 27.93 ±0.90      | 27.03 ±0.51      | 26.26 ±0.46           | 27.35 ±0.55           | 27.46 ±0.56           |

Note. Mean values followed by different letters, in the same row, differ significantly (P < 0.05). Mean values followed by different exponents, in the same column, differ significantly (P < 0.05).

| Table 3. Effect of ozone and heat treatment on the color parameter a* of strawberries during cold storage. |
|---------------------------------------------------------------|
| **Days of storage** | **Control** | **Control + HT** | **0.5 ppm ozone** | **1.0 ppm ozone** | **1.5 ppm ozone** | **0.5 ppm ozone + HT** | **1.0 ppm ozone + HT** | **1.5 ppm ozone + HT** |
|---------------------|-------------|-----------------|------------------|------------------|------------------|-----------------------|-----------------------|-----------------------|
| 0                   | 42.95 ±0.75 | 42.95 ±0.75     | 42.95 ±0.75      | 42.95 ±0.75      | 42.95 ±0.75      | 42.95 ±0.75           | 42.95 ±0.75           | 42.95 ±0.75           |
| 4                   | 44.95 ±0.55 | 42.72 ±0.42     | 45.66 ±0.46      | 45.95 ±0.95      | 44.33 ±0.53      | 44.54 ±0.44           | 45.46 ±0.66           | 45.48 ±0.48           |
| 8                   | 44.60 ±0.49 | 42.50 ±0.39     | 44.70 ±0.80      | 45.30 ±0.40      | 43.70 ±0.50      | 43.96 ±0.46           | 44.10 ±0.59           | 44.42 ±0.62           |
| 12                  | 44.32 ±0.72 | 42.36 ±0.66     | 43.97 ±0.67      | 44.71 ±0.51      | 43.06 ±0.60      | 43.42 ±0.42           | 42.80 ±0.61           | 43.45 ±0.55           |
| 16                  | 42.34 ±0.64 | 43.60 ±0.51     | 42.17 ±0.57      | 42.29 ±0.49      | 41.46 ±0.61      | 40.42 ±0.52           | 42.44 ±0.64           | 42.85 ±0.75           |

Note. Mean values followed by different letters, in the same row, differ significantly (P < 0.05). Mean values followed by different exponents, in the same column, differ significantly (P < 0.05).

| Table 4. Effect of ozone and heat treatment on the color parameter b* of strawberries during cold storage. |
|---------------------------------------------------------------|
| **Days of storage** | **Control** | **Control + HT** | **0.5 ppm ozone** | **1.0 ppm ozone** | **1.5 ppm ozone** | **0.5 ppm ozone + HT** | **1.0 ppm ozone + HT** | **1.5 ppm ozone + HT** |
|---------------------|-------------|-----------------|------------------|------------------|------------------|-----------------------|-----------------------|-----------------------|
| 0                   | 25.72 ±0.55 | 25.72 ±0.55     | 25.72 ±0.55      | 25.72 ±0.55      | 25.72 ±0.55      | 25.72 ±0.55           | 25.72 ±0.55           | 25.72 ±0.55           |
| 4                   | 26.65 ±0.60 | 24.97 ±0.80     | 28.48 ±0.70      | 28.88 ±0.59      | 26.33 ±0.53      | 27.86 ±0.56           | 27.85 ±0.65           | 28.02 ±0.42           |
| 8                   | 27.06 ±0.56 | 24.56 ±0.61     | 27.87 ±0.69      | 28.33 ±0.61      | 25.55 ±0.75      | 26.45 ±0.45           | 25.83 ±0.63           | 26.77 ±0.57           |
| 12                  | 27.31 ±0.51 | 24.22 ±0.42     | 26.72 ±0.41      | 27.60 ±0.41      | 24.37 ±0.57      | 25.87 ±0.67           | 24.79 ±0.59           | 25.92 ±0.69           |
| 16                  | 24.56 ±0.55 | 25.43 ±0.40     | 23.91 ±0.65      | 23.38 ±0.61      | 23.31 ±0.45      | 23.10 ±0.70           | 24.12 ±0.39           | 24.41 ±0.55           |

Note. Mean values followed by different letters, in the same row, differ significantly (P < 0.05). Mean values followed by different exponents, in the same column, differ significantly (P < 0.05).
In the present study, ozonation at 1.0 ppm and 1.5 ppm plus heat treatment caused an increase in the L* parameter compared to the control sample during cold storage. Aguayo et al. (2006) found higher L* values in ozonated tomatoes after 15 days of storage. Tiwari et al. (2009) also reported an increase in the L* parameter in ozonated tomatoes. Higher L* values were also found in ozonated orange juices at several ozone concentrations and exposure times (Tiwari et al., 2008).

An increase in the parameter a* was observed in all treatments up to the 4th day of storage, which can be attributed to the breakdown of chlorophyll. After the 4th day of storage parameter a* decreased. Heat-treated strawberries showed a slight increase in the parameter a* during cold storage. Strawberries treated with ozone at a concentration of 0.5 ppm showed no statistically significant change in a* parameter compared to control samples. Ozone-treated strawberries at a concentration of 1.0 ppm showed statistically significantly higher values (p < .05) of the a* parameter, whereas ozone-treated strawberries at a concentration of 1.5 ppm and 1.5 ppm plus heat treatment showed statistically significantly lower (p < .05) values of parameter a* compared to control samples during cold storage. This could be attributed to the high sensitivity of carotenoids to ozone due to their high degree of unsaturation. Color parameter a* is affected by changes in the content of chlorophyll, lycopene, and anthocyanidins (Li et al., 2019).

In the present study heat treated, 0.5 ppm and 1.0 ppm ozone-treated strawberries showed a higher a* value compared to the control sample during cold storage. In contrast, ozone treatment at 1.5 ppm and 1.5 ppm plus heat treatment caused a statistically significant (p < .05) decrease in the parameter a* compared to the control samples. The lower value of the parameter a* could be attributed to the oxidizing effect of ozone on unsaturated compounds such as anthocyanins (Tiwari et al., 2009). Aguayo et al. (2006) did not report significant changes in lycopene content in whole and sliced ozone-treated tomatoes. Furthermore, other researchers reported that ozone did not affect the anthocyanin content of strawberries and blackberries, respectively (Keutgen and Pawelzik, 2008; Barth et al., 1995).

An increase in the b* parameter was observed in all treatments up to the 8th day of storage, which can also be attributed to the breakdown of chlorophyll. After the 8th day of storage b* parameter decreased. Strawberries treated with ozone at a concentration of 1.5 ppm plus heat treatment presented statistically significantly lower (p < .05) values of b* parameter compared to control samples during cold storage. Color parameter b* is affected by changes in the content of chlorophyll, carotenoids, and flavonoids (Lu et al., 2020).

Aguayo et al. (2006), found a slight decrease in the b* parameter in ozone-treated tomatoes at a concentration of 4 μL/L on the 15th day of storage. Barth et al. (1995), did not report significant changes in the b* parameter in ozone-treated berries at a concentration of 0.3 ppm. Tiwari et al. (2009), reported a decrease in the b* parameter in tomatoes by increasing the ozone concentration, while similar results were found in a previous study of them in orange juice (Tiwari et al., 2008).

**Sensory Analysis**

The results of sensory analysis are shown in Figure 6a-6c. The main sensory analysis that characterizes a food and determines its acceptance by consumers relates to appearance, taste, and odor. Appearance has a predominant role because an extremely repulsive appearance will reduce the acceptability of a food without further effort by consumers to taste and smell it. The degradation of the aforementioned sensorial characteristics over a period of time is the result of physicochemical, biochemical and microbiological changes occurring in a food.

The unprocessed strawberries kept their appearance at acceptable levels until the 8th day of storage. Heat-treated, ozone-treated strawberries at a concentration of 0.5 ppm and at a concentration of 0.5 ppm plus heat treatment showed a slight better appearance that was maintained at acceptable levels until the 10th day of storage. Treatment with ozone at concentrations of 1.0 ppm and 1.5 ppm and at the same concentrations in combination with heat treatment caused an acceleration of the appearance deterioration which remained acceptable for a period of less than 8 days. The main causes responsible for the deterioration of the appearance are enzymatic browning reactions, weight loss, surface growth of fungi, and mechanical injuries. The degraded taste and odor is mainly due to undesirable microbial fermentations.
Heat-treated, ozone-treated strawberries at 0.5 ppm and at 0.5 ppm plus heat treatment showed a slightly better appearance compared to the control samples. This could be attributed to the destruction of microorganisms by the ozone and heating as well as the inactivation of the enzymes that catalyze browning reactions. In contrast, treatment with ozone at 1.0 ppm and 1.5 ppm and at the same concentrations plus heat treatment increased the deterioration rate of the appearance due to the high oxidative capacity of ozone and the high perishability of strawberry.
Untreated strawberries kept their taste at acceptable levels until the 12th day of storage. Heat-treated and ozone-treated strawberries at a concentration of 0.5 ppm showed a slightly better taste and kept their taste acceptable for a period of more than 12 days. Ozone treatment at concentrations of 1.0 ppm and 1.5 ppm caused acceleration of taste deterioration, which remained acceptable until day 8 of storage. Strawberries treated with ozone at 1.0 ppm and 1.5 ppm plus heat treatment kept their taste acceptable for a period of less than 8 days.

Heat-treated and ozone-treated strawberries at 0.5 ppm showed a slightly better taste compared to the control sample. Ozone treatment at 1.0 ppm and 1.5 ppm caused extended oxidative changes leading to lower levels of taste compared to untreated strawberries. The same results were also observed in the case of ozone-treated strawberries at 1.0 ppm and 1.5 ppm plus heat treatment. The lower levels of taste in ozone-treated strawberries at the above concentrations could be attributed to the formation of organic acids due to the oxidation of unsaturated organic compounds by ozone.

Untreated strawberries and ozone-treated strawberries at all concentrations showed a musty odor at 16th day of sold storage, whereas heat-treated strawberries showed a much less intense musty odor. This could be attributed to the thermal inactivation of enzymes responsible for the formation of volatile compounds by fermentation reactions.

**Conclusion**

The results of the present study showed that certain physicochemical characteristics of strawberries such as weight loss, titratable acidity, and TSS content were significantly altered in all treatments applied, compared to the control sample during cold storage. The appearance of the strawberries was maintained at acceptable levels until the 8th day of storage while their taste was maintained at acceptable levels until the 12th day of storage. Based on the general appearance, which is the dominant criterion for determining the selection of a product as consumable, the application of heat treatment, ozone at 0.5 ppm, and ozone at 0.5 ppm plus heat treatment contributed to the extension of strawberry’s shelf life to 10 days, while the shelf life for unprocessed strawberries was 8 days. On the contrary, the application of ozone at 1.0 ppm, 1.5 ppm, 1.0 ppm plus heat treatment and 1.5 ppm plus heat treatment reduced the shelf life of the product to 6 days. However, ozonation cannot prevent the appearance of musty odor on the last day of storage, which is partially prevented by heat treatment. In our opinion, even a small extension of the shelf life of this perishable product can have a positive impact on its commercial value.

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**Disclosure statement**

The authors have no conflict of interest to report.

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