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Strawberry Variety Influences the Effectiveness of Postharvest Treatment with Gaseous Ozone: Impact on the Physicochemical, Microbiological, and Bioactive Properties of the Fruit

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Abstract: The aim of this research was to evaluate the influence of strawberry variety on the effectiveness of ozone application on the harvested fruit during 12 days of storage. Batches (400 g) of strawberries of the San Andreas (SA) and Camino Real (CR) varieties were stored at 10 ± 0.2 °C and exposed to gaseous ozone (0, 0.3, and 1.0 ppm) for 24 h. After the ozone exposure, the strawberries continued to be stored under refrigeration until the end of the experiment. Samples were taken daily and measurements were carried out on their physicochemical properties (weight loss, hardness, color, pH, and total soluble solids), microbiological profile (mesophilic aerobes, molds, and yeasts), bioactive compounds (total phenolic compounds and total anthocyanins), and antioxidant capacity. The obtained experimental kinetics were modeled using a first-order kinetic model. Independent of the strawberry variety, the 0.3 ppm ozone treatment generally showed the best results for most of the quality parameters evaluated. On the other hand, strawberries exposed to 1.0 ppm suffered some negative effects on fruit preservation, mainly regarding their physicochemical properties. Importantly, the CR variety presented less negative effects of gaseous ozone application compared to SA, especially in terms of weight loss, color, hardness, and anthocyanins.

Keywords: antioxidant capacity; berries; food safety; non-thermal technologies; quality

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1. Introduction

The strawberry (*Fragaria* × *ananassa*) is considered a fruit with a high content of moisture, sugars, vitamins, and minerals [1]. In addition, it has been reported that, when consumed, this fruit has beneficial health effects due to its nutraceutical properties, which are attributed to its high polyphenol content and antioxidant activity [2]. These properties and its flavor have caused an increase in strawberry consumption in the global population [3]. However, despite the characteristics already mentioned, the strawberry is a fruit with a short shelf life [4].

Strawberry shelf life may depend on the cultivar type; however, it can be 1 to 2 days under ambient conditions and 5 to 7 days under refrigeration [2]. In addition, the strawberry is very susceptible to mechanical damage; likewise, it has high metabolic and microbial activity due to its high moisture content [5]. For this reason, the food industry is in a constant search for innovative, profitable, and scalable postharvest treatments that would allow for extending the shelf life of strawberries [6]. Thus, hurdle technologies, such as thermal treatments, refrigeration, modified atmospheres, and non-thermal technologies, among others, have been studied in recent years and implemented for the conservation of this fruit [7].

Since ozone was declared a GRAS (generally recognized as safe) compound in the mid-1990s [8], the food industry has shown interest in developing processes that involve its applications. Within non-thermal technologies, ozone exposure may be a viable option for the postharvest treatment of berries, especially for strawberries [9,10]. The effectiveness of ozone against spoilage microorganisms in the strawberry is mainly due to its effect against Gram-negative and Gram-positive bacteria, spores, and vegetative cells. In this sense, ozone has an oxidative effect directly on the glycoproteins and glycolipids of the bacterial membrane [6]. In addition, ozone allows for the preservation of organoleptic properties, the deodorization of the environment to avoid transmission of undesirable odors, and the reduction in moisture loss in the fruit [11]. Due to all of these characteristics, ozone represents a promising postharvest technology that could prolong the shelf life of strawberries [12].

Currently, there is research where the strawberry has been successfully subjected to ozone concentrations ranging from 0.1 ppm to 8 ppm in different treatment times [9,13,14]. However, it has been shown that high ozone concentrations and prolonged exposure times to this gas can cause important changes in the quality of the strawberry. In addition, for the application at an industrial level, it is important to understand how the effectiveness of gaseous ozone can vary depending on the variety of the strawberry that is being treated. The aim of this work was to evaluate the influence of strawberry variety on the effectiveness of application of different ozone concentrations on the harvested fruit, measuring its physicochemical, bioactive, and microbiological properties during 12 days of storage. To reach this objective, two strawberry varieties with different shelf lives without treatment were selected. In this sense, according to strawberry producers, the Camino Real (CR) variety is more perishable compared to the San Andreas (SA) variety. In this work, we aim to study how this distinction between these two varieties translates into differences in the response to postharvest ozone treatment.

2. Materials and Methods

2.1. Raw Material and Treatments with Gaseous Ozone

The strawberries of the CR and SA varieties (Figure 1), with a ripeness degree of 6 (uniform size and intense red color) in accordance with the Mexican Standard NMX-FF-062-SCFI-2002 [15], were collected in orchards in the municipality of Irapuato (Mexico). Immediately, the strawberries were transported to the laboratory for their treatment and analysis. Subsequently, for each treatment, clamshell containers were filled with 400 ± 7 g of strawberries (two containers per treatment).

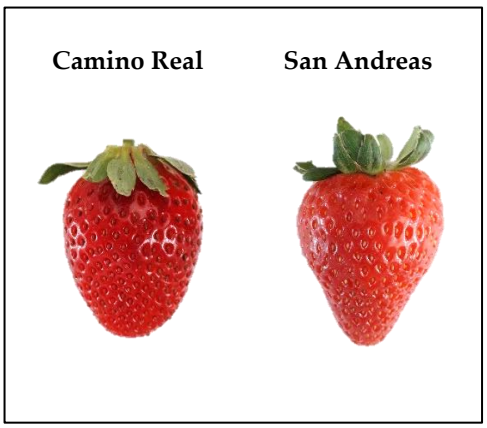

Figure 1. Strawberry varieties (*Fragaria* × *ananassa*) studied in this research.

The strawberries of both varieties (CR and SA) were continuously exposed to gaseous ozone for 24 h through a refrigerated chamber (temperature: 10 ± 0.2 °C and volume: 487,760 cm^3) adapted with a gaseous ozone generator developed by the company Ozone Carbar's, Mexico. The ozone concentrations evaluated were 0 ppm (CR0.0 and SA0.0),

0.3 ppm (CR0.3 and SA0.3), and 1 ppm (CR1.0 and SA1.0). Subsequently, the samples were stored at 10 ± 0.2 °C for 12 days. During this period, changes in physicochemical (weight loss, hardness, color, pH, and total soluble solids), bioactive (total phenolic compounds, total anthocyanins, and antioxidant capacity), and microbiological (mesophilic aerobic bacteria, molds, and yeasts) properties were evaluated in the fruit.

2.2. Determination of Physicochemical Properties

2.2.1. Weight Loss

During the 12 days of storage, six strawberries (for each treatment replicate) were individually weighed using an analytical balance (PioneerTM, OHAUS, Parsippany, NJ, USA). The percentage of weight loss was calculated using Equation (1):

$$\text{Weight loss } (\%) = \frac{\text{Weight } (g)}{\text{Initial weight } (g)} \times 100 \quad (1)$$

2.2.2. Hardness

Hardness was determined at the strawberry equator using a texturometer (CT3TM, AMETEK Brookfield, Chandler, AZ, USA) with a cylindrical probe of 2 mm in diameter, a puncture speed of 1 mm/s, a distance of 3 mm, and a 6 mm thickness. The results were expressed in Newtons (N) and, subsequently, the percentage of hardness loss was calculated using Equation (2) [16].

$$\text{Hardness loss } (\%) = \frac{\text{Hardness } (g)}{\text{Initial hardness } (g)} \times 100 \quad (2)$$

2.2.3. Color

Color measurements were taken on the same surface of six different strawberries using a colorimeter (ColorFlexEZ, HunterLab, Reston, VA, USA). For each treatment, the color analysis was carried out using independent duplicates. The results were expressed according to the CIEL*a*b* system; the determined parameters were L* (luminosity, L* = 0 (black) and L* = 100 (white)), a*, and b* (opposite color dimensions, (a* negative values = green and a* positive values = red, b* negative values = blue and b* positive values = yellow)) [17]. For the calculation of chroma or intensity, hue angle or hue, and color differential parameters, Equations (3), (4), and (5) were used, respectively.

$$\text{Chroma} = \sqrt{(a^*)^2 + (b^*)^2} \quad (3)$$

$$\text{Hue angle} = \tan^{-1}\left(\frac{b^*}{a^*}\right) \quad (4)$$

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2};\ \Delta L = L - L_0;\ \Delta a = a - a_0;\ \Delta b = b - b_0 \quad (5)$$

where L_0, a_0, and b_0 represent the values of the L*, a*, and b* strawberry coordinates without treatment (t = 0 days).

The percentage of chromatic property loss (lightness, chroma, and hue angle) was calculated using Equation (6):

$$\text{Chromatic properties } (\%) = \frac{\text{Chromatic value}}{\text{Initial chromatic value}} \times 100 \quad (6)$$

2.2.4. pH and Total Soluble Solids

The pH was measured using a dilution of strawberry and distilled water (1:10 w/v) using a potentiometer (Orion Star A214, Thermo Fisher Scientific, Waltham, MA, USA) [18]. On the other hand, the total soluble solids (TSSs) were determined using a refractometer

(Hi 96801, HANNA Instruments, Woonsocket, RI, USA) at a temperature of 20 ± 1 °C. The results were expressed in °Brix [4].

2.3. Determination of Bioactive Compounds and Antioxidant Capacity

2.3.1. Extraction of Bioactive Compounds

To obtain a hydroalcoholic extract, the methodology proposed by Chordi-Barrufet [19] was used with some modifications. A 10 mL aliquot of methanol (80% v/v) was added to 5 g (fresh sample, moisture: 89.90 ± 0.32%) of crushed strawberry sample and stirred for 1 h in the absence of light. Finally, the mixture was centrifuged at 3500 rpm for 15 min and filtered for the subsequent bioactive compound determinations.

2.3.2. Total Phenolic Compounds

Total phenolic compound (TPC) content in strawberries was determined following the methodology proposed by Singleton et al. [20]. A 20 µL aliquot of the hydroalcoholic extract was added with 250 µL of the 1 N Folin–Ciocalteu reagent, allowing it to rest for 8 min in the dark. Then, 1250 µL of sodium carbonate (7.5%) and 480 µL of distilled water were added and it was stirred at 150 rpm for 1 h. Subsequently, samples were left to rest for 30 min in the dark. Finally, the samples were read at 760 nm in a UV–Vis spectrophotometer (GENESYS 10S, Thermo ScientificTM, Waltham, MA, USA). The results were expressed in milligrams of gallic acid equivalent per 100 g of fresh sample (mg GAE/100 g FW).

2.3.3. Total Anthocyanin Content

Total anthocyanin content (TAC) was measured following the pH differential methodology proposed by Lee et al. [21]. A quantity of 500 µL of the hydroalcoholic extract was taken and 1750 µL of pH 1 buffer was added. On the other hand, 1750 µL of pH 4.5 buffer were added to 500 µL of the hydroalcoholic extract. Both mixtures were left to react for 15 min in the dark. Finally, the samples were read at 520 nm and 700 nm using a UV–Vis spectrophotometer (GENESYS 10S, Thermo ScientificTM, Waltham, MA, USA). TAC was calculated and expressed in terms of mg of cyanidin-3-glucoside per 100 g of fresh sample (mg C3G/100 g FW) using Equation (7):

$$\text{TAC} = \frac{(\text{A})(\text{MW})(\text{DF})(\text{EV})(1000)}{(\varepsilon)(1)(\text{M})};\ \text{A} = (\text{A}_{510} - \text{A}_{700})_{\text{pH1}} - (\text{A}_{510} - \text{A}_{700})_{\text{pH4.5}} \quad (7)$$

where MW is the molecular weight of cyanidin-3-glucoside (449 g/mol), DF is the dilution factor, EV is the extract volume, ε is the molar extinction coefficient of cyanidin-3-glucoside, and M is the mass of the strawberries.

2.3.4. Antioxidant Capacity

The antioxidant capacity (AC) was determined following the 2,2-diphenyl-1-pricrylhydrazyl (DPPH) radical inhibition methodology described by Brand-Williams et al. [22]. A 3 mL aliquot of DPPH solution (6.1×10^{-5} M in methanol) was reacted in 100 µL of the hydroalcoholic extract of the sample. The mixture was left to rest in the dark for 30 min and, subsequently, the sample reading was carried out at 515 nm using a UV–Vis spectrophotometer (GENESYS 10S, Thermo ScientificTM, Waltham, MA, USA). The AC was expressed from the percentage of inhibition of the DPPH radical (Equation (8)):

$$\%\ \text{Inhibition} = \frac{(\text{Abs}_0 - \text{Abs}_1)}{\text{Abs}_0} \times 100 \quad (8)$$

where Abs_0 is the absorbance of the DPPH radical before the reaction and Abs_1 is the absorbance of the DPPH mixture with the sample.

2.4. Determination of Microbiological Properties

2.4.1. Molds and Yeasts

The total count of molds and yeasts was carried out following the methodology proposed by the Official Mexican Standard NOM-111-SSA1-1994 [23]. An aliquot of 1 mL of each decimal dilution of the samples was placed in a Petri dish and then 20 mL of the PDA culture medium was added. Finally, the samples were incubated at 25 ± 1 °C. Colony counting was performed on days 3, 4, and 5 of incubation. The results were expressed as log10 cfu/mL.

2.4.2. Aerobic Mesophilic Bacteria

The count of aerobic mesophilic bacteria (AMB) was carried out following the methodology proposed by the Official Mexican Standard NOM-092-SSA1-1994 [24]. A 1 mL aliquot of each decimal dilution of the samples was placed in a Petri dish, then 20 mL of Standard Method Agar was added. Finally, the samples were incubated at 35 ± 2 °C for 48 ± 2 h. Colony counting was performed on days 3, 4, and 5 of incubation. The results were expressed as log10 cfu/mL.

2.5. Kinetics Modeling

The modeling of the kinetics of weight loss, hardness, and chromatic properties was carried out using a first-order kinetics model (Equation (9)) [25].

$$X = X_e + (X_0 - X_e)e^{-kt} \quad (9)$$

where X is the value of the attribute generated by the model, X_e represents the value of the attribute at equilibrium, X_0 represents the initial value of the attribute, k represents the rate constant of the reaction (min^{-1}), and t represents the time of the reaction (days).

Likewise, the generalized reduced gradient (GRG) optimization method was used, which reduces the minimum value of the variables that provide a solution to the equation.

2.6. Statistical Analysis

A three-way ANOVA was performed to evaluate the effect of the independent variables (strawberry variety: CR and SA, ozone concentration: 0, 0.3, and 1 ppm, and storage time: 1–12 days) on the physicochemical, bioactive, and microbiological properties of the strawberry. The first-order model constants (k and X_{eq}) were analyzed using a two-way ANOVA (strawberry variety and ozone concentration). For F-tests significant at $p < 0.05$, post hoc Tukey tests were conducted. All statistical analyses were performed using SPSS 18.0 software (SPSS Inc., Chicago, IL, USA).

3. Results and Discussion

3.1. Physicochemical Properties

3.1.1. Weight Loss

Figure 2A,B shows the effect of the ozone concentration and the strawberry variety on the percentage of fruit weight loss during storage. In general terms, in both strawberry varieties, storage time significantly ($p < 0.05$) affected weight loss (Table 1). Regarding the influence of ozone concentration, the effect of ozone on weight loss was more pronounced as the storage period increased, but only for the CR variety. For example, on day 12, the samples that were not treated with ozone (CR0.0) lost 10% more weight ($p < 0.05$) with respect to the ozonated samples (CR0.3 and CR1.0) (Figure 2A). Several studies show that ozone improves the prevention of weight loss in fruits [4,26,27]. This effect can be attributed to the fact that ozone delays fruit transpiration, which causes a decrease in moisture loss [26].

Table 1. The results of three-way ANOVAs for weight loss, color properties (lightness, chroma, hue angle, and total color difference: ΔE), pH, and total soluble solids (TSSs) of strawberries. The main effects and interactions of ozone concentration, strawberry variety, and storage time (significant results in bold).

	Weight loss		**Hardness**		**Lightness**		**Chroma**		**Hue angle**		**ΔE**		**pH**		**TSSs**	
	F	***p***	**F**	***p***	**F**	***p***	**F**	***p***	**F**	***p***	**F**	***p***	**F**	***p***	**F**	***p***
MAIN EFFECT																
Ozone concentration (A)	0.196	0.822	**6.610**	**0.002**	**8.161**	**0.001**	1.475	0.236	2.154	0.124	1.384	0.258	**8.088**	**0.000**	2.173	0.116
Strawberry variety (B)	3.105	0.083	**50.259**	**0.000**	**40.268**	**0.000**	**107.260**	**0.000**	**38.527**	**0.000**	0.250	0.619	**34.357**	**0.000**	**69.671**	**0.000**
Storage time (C)	**719.344**	**0.000**	**18.337**	**0.000**	**231.219**	**0.000**	**215.272**	**0.000**	**14.980**	**0.000**	**283.658**	**0.000**	1.197	0.315	**2.382**	**0.030**
INTERACTIONS																
A × B	**4.234**	**0.019**	**10.648**	**0.000**	1.047	0.357	1.112	0.335	0.380	0.685	**8.278**	**0.001**	2.401	0.094	1.610	0.202
A × C	**7.184**	**0.000**	1.118	0.345	0.506	0.788	1.262	0.259	0.936	0.517	1.838	0.070	1.306	0.245	1.088	0.372
B × C	**3.530**	**0.040**	0.414	0.870	2.446	0.071	0.303	0.890	1.753	0.099	0.957	0.432	0.907	0.461	0.838	0.542
A × B × C	1.597	0.189	0.266	0.994	1.080	0.375	1.220	0.283	1.379	0.163	1.347	0.220	1.079	0.381	0.778	0.673

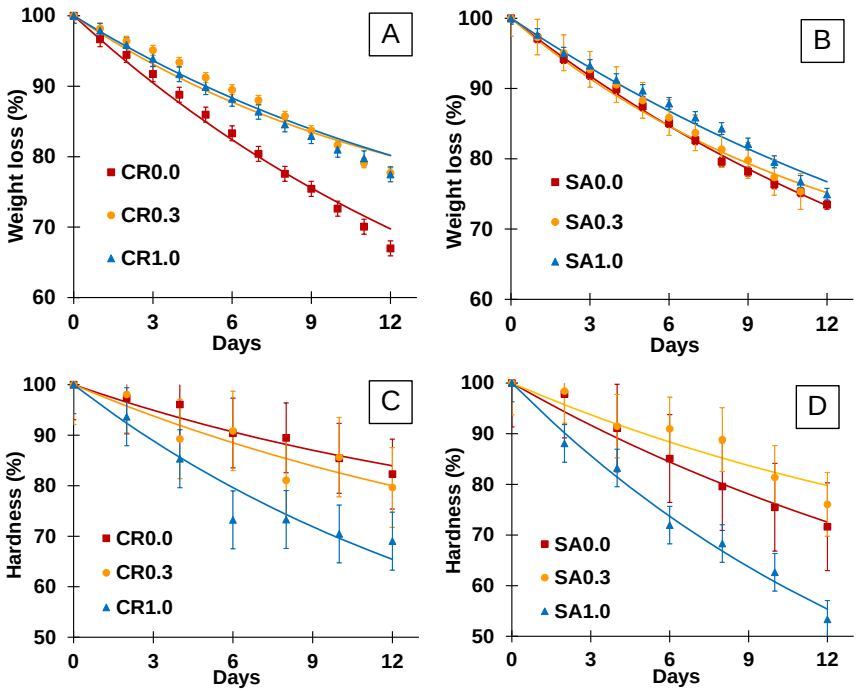

Figure 2. The effect of strawberry variety (CR: Camino Real and SA: San Andreas) and ozone concentration (0.0: 0 ppm, 0.3: 0.3 ppm, and 1.0: 1 ppm) on weight loss (**A**,**B**) and hardness (**C**,**D**) of fruit during 12 days of storage (10 ± 0.2 °C). First-order kinetic model is represented by lines (for CR and SA varieties) colored in red (0.0: 0 ppm), yellow (0.3: 0.3 ppm), and blue (1.0: 1 ppm). Error bars represent 95% confidence intervals.

For both strawberry varieties and all studied treatments, the experimental data of the percentage of weight loss correctly adjusted ($R^2 > 0.96$) to a first-order kinetics model (Table 2). The rate constant, k, was affected ($p < 0.05$) by the strawberry variety. In this sense, CR showed a higher k value than SA. This fact shows that the CR variety is more susceptible to weight loss than SA. Regarding the equilibrium constant, X_{eq}, it was affected by the strawberry variety and the ozone concentration. Thus, the CR variety showed a significantly higher X_{eq} value ($p < 0.05$) in comparison to SA. In the case of ozone concentration, the X_{eq} values for the treatments without ozonation were lower ($p < 0.05$) with respect to the values reached for the treatments with ozone. This result indicates that ozone contributed to avoiding weight loss in the fruit.

Table 2. First-order kinetic parameters (k and X_{eq}) for weight loss, hardness, lightness, chroma, and total color difference (ΔE) of strawberries of San Andreas (SA) and Camino Real (CR) varieties treated with different ozone concentrations (0.0: 0 ppm, 0.3: 0.3 ppm, and 1.0: 1 ppm).

Parameter	Treatment	k ($\times 10^{-2}$) [min^{-1}]	X_{eq}	R^2
Weight loss	CR.00	5.50 ± 0.70^{ax}	41.52 ± 1.55^{ax}	0.99
	CR0.3	4.75 ± 0.35^{ax}	62.18 ± 2.83^{cx}	0.96
	CR1.0	4.50 ± 0.00^{ax}	55.02 ± 1.76^{bx}	0.99
	SA0.0	4.50 ± 0.00^{ay}	35.65 ± 1.84^{ay}	0.99
	SA0.3	4.75 ± 0.35^{ay}	55.78 ± 3.58^{cy}	0.98
	SA1.0	4.00 ± 0.00^{ay}	36.45 ± 1.48^{by}	0.97
Hardness	CR0.0	4.50 ± 0.00^{ax}	60.87 ± 11.80^{by}	0.91
	CR0.3	4.50 ± 0.70^{ax}	51.48 ± 6.37^{by}	0.67
	CR1.0	5.25 ± 0.35^{ax}	25.83 ± 8.59^{ay}	0.87
	SA0.0	4.00 ± 1.06^{ax}	25.86 ± 6.91^{bx}	0.80
	SA0.3	5.00 ± 0.00^{ax}	55.14 ± 0.70^{bx}	0.88
	SA1.0	6.50 ± 0.70^{ax}	18.11 ± 2.36^{ax}	0.83

Table 2. *Cont.*

Parameter	Treatment	k ($\times 10^{-2}$) [min^{-1}]	X_{eq}	R^2
Lightness	CR0.0	10.00 ± 0.00^{ax}	70.63 ± 8.16^{ax}	0.94
	CR0.3	12.25 ± 3.88^{ax}	77.88 ± 10.92^{ax}	0.95
	CR1.0	15.00 ± 3.07^{ax}	82.94 ± 11.13^{ax}	0.95
	SA0.0	15.00 ± 3.07^{ax}	77.92 ± 2.33^{ax}	0.94
	SA0.3	15.00 ± 3.07^{ax}	73.98 ± 10.08^{ax}	0.98
	SA1.0	20.00 ± 0.00^{ax}	81.92 ± 2.66^{ax}	0.99
Chroma	CR0.0	22.50 ± 5.31^{ax}	66.47 ± 10.11^{ax}	0.91
	CR0.3	12.50 ± 3.53^{ax}	56.04 ± 14.82^{ax}	0.86
	CR1.0	9.50 ± 0.70^{ax}	56.46 ± 14.38^{ax}	0.93
	SA0.0	17.00 ± 5.81^{ax}	69.92 ± 2.89^{ax}	0.93
	SA0.3	19.50 ± 7.45^{ax}	66.16 ± 12.94^{ax}	0.94
	SA1.0	20.00 ± 7.07^{ax}	67.68 ± 4.79^{ax}	0.94
ΔE	CR0.0	25.00 ± 0.00^{bx}	14.80 ± 0.62^{ax}	0.93
	CR0.3	25.00 ± 7.07^{abx}	16.67 ± 1.08^{ax}	0.91
	CR1.0	10.00 ± 0.00^{ax}	13.75 ± 0.30^{ax}	0.95
	SA0.0	15.00 ± 0.00^{bx}	15.37 ± 0.11^{ax}	0.92
	SA0.3	12.50 ± 3.53^{abx}	14.74 ± 0.10^{ax}	0.83
	SA1.0	20.00 ± 0.00^{ax}	15.40 ± 0.23^{ax}	0.92

Different superscripts between rows of the same column indicate a significant difference ($p < 0.05$) between treatments according to the Tukey test, where a, b, and c are dependent on the ozone concentration and x and y are dependent on the variety.

3.1.2. Hardness

Figure 2C,D shows the effect of the ozone concentration and the strawberry variety on the percentage of fruit hardness loss during storage. The initial hardness values were 0.57 ± 0.01 N and 0.52 ± 0.06 N for the CR and SA varieties, respectively. These values represent 100% of the hardness loss figures (Figure 2C,D). In general, for both strawberry varieties, the days of storage had a significant influence ($p < 0.05$) on this parameter (Table 1). In this sense, by increasing the time of storage, the hardness of the strawberry decreased in all treatments. Regarding the effect of ozone concentration, the exposure of both strawberry varieties to 1 ppm caused the greatest decrease in hardness compared to the other treatments. However, this effect was more pronounced in the SA variety compared to CR. For example, on day 12, the SA1.0 samples lost up to 47% of their hardness compared to the CR1.0 samples, which lost up to 31% of their hardness. Several studies show that high ozone concentrations can cause damage to the structural components of the plant cell wall, especially to lignin and proteins [28,29]. These damages at the cellular level can impact the macroscopic characteristics of the fruit, and such is the case for hardness.

The experimental data of the hardness loss percentage were fitted ($R^2 > 0.67$) to a first-order kinetics model (Table 2). k was not affected by the strawberry variety or by the ozone concentration. However, X_{eq} was affected by both factors. Thus, the treatments of 1 ppm ozone showed lower X_{eq} values ($p < 0.05$) compared to the rest of the treatments. This indicates that samples treated at this concentration are more susceptible to a loss in hardness compared to samples ozonated at 0.3 ppm and those that were not treated with ozone. Likewise, the X_{eq} of SA was significantly ($p < 0.05$) lower than that of CR. This trend indicates that the SA variety is more susceptible to losing hardness during storage compared to the CR variety.

3.1.3. Color

Figure 3 shows the effect of the ozone concentration and the strawberry variety on the chromatic properties of the fruit during storage. The initial luminosity values were 32.79 ± 1.13 and 37.06 ± 1.77 for the CR and SA varieties, respectively. These values represent 100% of the luminosity loss figures (Figure 3A,B).

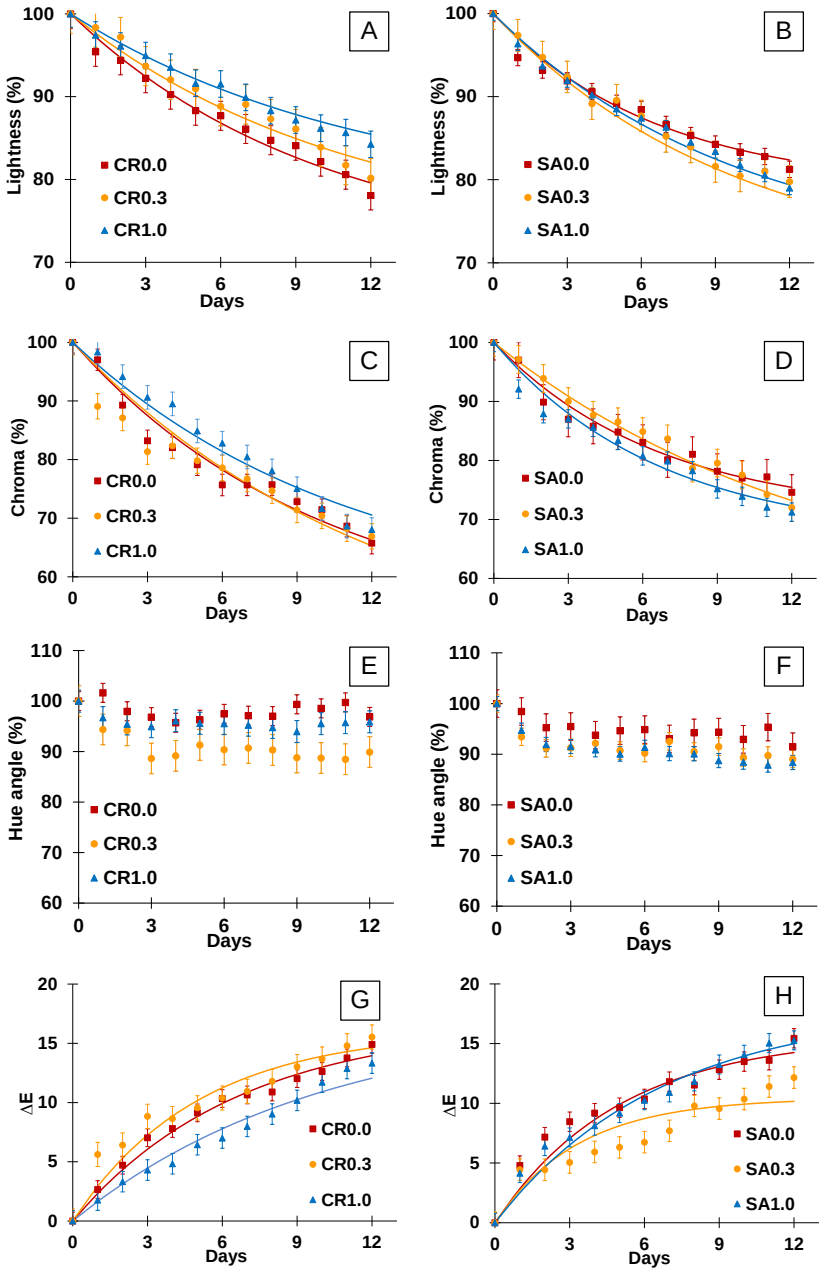

Figure 3. The effect of strawberry variety (CR: Camino Real and SA: San Andreas) and ozone concentration (0.0:0 ppm, 0.3:0.3 ppm, and 1.0:1 ppm) on lightness (**A**,**B**), chroma (**C**,**D**), hue angle (**E**,**F**), and the total color difference (**G**,**H**) of fruit during 12 days of storage (10 ± 0.2 °C). First-order kinetic model is represented by lines (for CR and SA varieties) colored in red (0.0:0 ppm), yellow (0.3:0.3 ppm), and blue (1.0:1 ppm). Error bars represent 95% confidence intervals.

For the luminosity values, the three variables studied (storage time, ozone concentration, and strawberry variety) had a significant influence (Table 1). Thus, by increasing the time of storage, the luminosity of the strawberries decreased. Regarding the ozone concentration, in the CR variety, the higher the ozone concentration, the less the luminosity loss in the fruit. In this sense, it has been reported that ozone has a bleaching effect, which makes strawberries exposed to this gas less susceptible to a loss in luminosity [13]. On the contrary, in the SA variety, the application of ozone caused a greater loss in luminosity in the fruits. Panou et al. [4] reported that using high concentrations of ozone can cause superficial lesions in the cell wall of the fruit, which can affect the chromatic properties of the fruit.

The experimental data of the percentage of luminosity loss were correctly fitted ($R^2 > 0.94$) to a first-order kinetics model (Table 2). The model constants, k and X_{eq}, were not affected by the strawberry variety or by the ozone concentration (Table 2).

In the case of chroma or color intensity of the fruit, the initial values were 37.51 ± 0.97 and 45.39 ± 1.89 for the CR and SA varieties, respectively. These values represent 100% of the chroma loss figures (Figure 3C,D). For the experimental data of chroma, the storage time and the strawberry variety significantly affected this parameter (Table 1). Likewise, as the storage time increased, the chroma for all of the samples decreased. However, for the last storage day, the CR variety had a loss in chroma of 33%, and this value was higher compared to the decrease presented by the SA variety (27%). The experimental data of the percentage of chroma loss were correctly fitted ($R^2 > 0.86$) to a first-order kinetics model (Table 2). The model constants, k and X_{eq}, were not affected by the strawberry variety or by the ozone concentration (Table 2).

Regarding the hue or tone angle, the initial values of this parameter were 29.44 ± 0.83 and 34.63 ± 1.05 for the CR and SA varieties, respectively. These values represent 100% of the hue angle loss figures (Figure 3E,F). For the experimental data of hue, the storage day and the strawberry variety were affected significantly (Table 1). Thus, by increasing the storage time, the hue angle showed slight decreases with respect to its initial value. For this reason, the experimental data could not be adjusted to the first-order kinetics model. Regarding the strawberry variety, the SA variety presented higher hue angle values during storage compared to the CR variety.

Finally, for the color difference (Figure 3G,H), the storage time caused significant changes in this parameter (Table 1). These changes were modulated by the ozone concentration and the strawberry variety (Table 1). Thus, in the SA variety, it was observed that the application of ozone at a concentration of 0.3 ppm contributed to avoiding global changes in the color of the fruit in comparison with the treatments of 1 ppm and without ozone application (Figure 3H). In this sense, some authors report that high ozone concentrations can cause a breakage in the aromatic rings of anthocyanins. This fact can cause oxidation and degradation of the strawberry pigments that are responsible for its characteristic color [13,30].

The experimental data of the color difference were fitted correctly ($R^2 > 0.83$) to a first-order kinetics model (Table 2). X_{eq} was not affected by the strawberry variety or by the ozone concentration. However, k was affected by the ozone concentration ($p < 0.05$). Thus, for both varieties, the samples that were not ozonated presented higher k values with respect to the samples exposed to 1 ppm of ozone. In this sense, several authors mention that the differences in the color of strawberries subjected to ozonation are directly associated with the ability of ozone to chemically inhibit color changes [13,18].

3.1.4. pH and Total Soluble Solids

Figure 3 shows the effect of ozone concentration and strawberry variety on pH and total soluble solids (TSSs) during storage. In the case of pH, the initial values were 3.89 ± 0.14 and 3.65 ± 0.03 for CR and SA, respectively. The strawberry variety had a significant effect ($p < 0.05$) on the pH during storage (Table 1). In this sense, the SA variety presented lower pH values compared to CR (Figure 4A,B). Regarding ozone concentration, this variable significantly affected the pH of both strawberry varieties (Table 1). However, these changes were more pronounced in the SA variety as the days of storage increased (Figure 4B).

For the TSSs, the initial values were 9.40 ± 0.12 °Brix and 7.94 ± 0.49 °Brix for CR and SA, respectively. Akšić et al. [31] report that 99% of strawberry TSSs are composed mainly of glucose, sucrose, and fructose. The variables of strawberry variety and storage time had a significant effect on the TSSs (Table 1). In this sense, the CR variety presented higher TSS values during storage compared to the SA variety (Figure 4C,D).

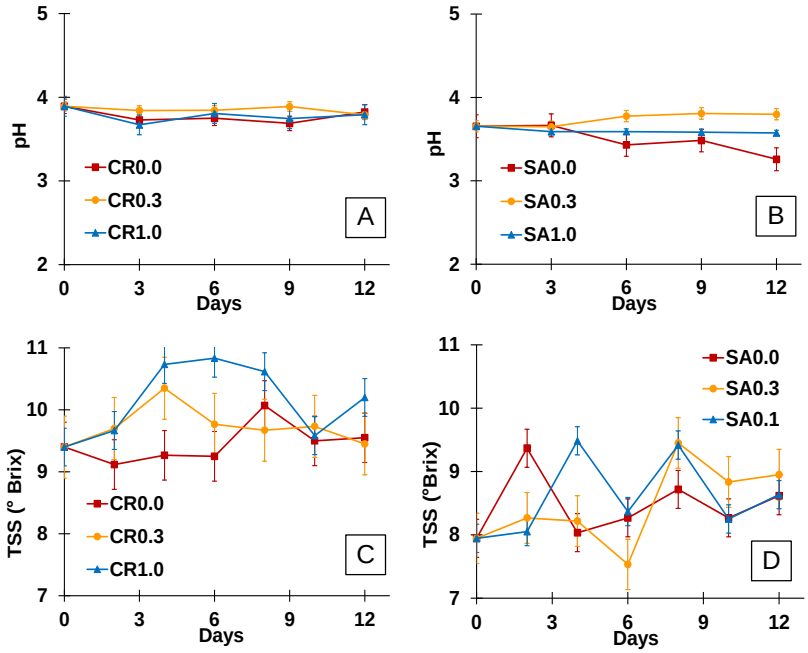

Figure 4. The effect of strawberry variety (CR: Camino Real and SA: San Andreas) and ozone concentration (0.0:0 ppm, 0.3:0.3 ppm, and 1.0:1 ppm) on pH (**A**,**B**) and total soluble solids (**C**,**D**) of fruit during 12 days of storage (10 ± 0.2 °C). Error bars represent 95% confidence intervals.

3.2. Bioactive Compounds and Antioxidant Capacity

Figure 5 shows the effect of ozone concentration and strawberry variety on the concentration of bioactive compounds and antioxidant capacity of the fruit during storage. The initial content of the total phenolic compounds (TPCs) was 330.83 ± 43.40 mg GAE/100 g FW and 338.11 ± 35.58 mg GAE/100 g FW for the CR and SA varieties, respectively. The TPC values are similar to those reported by other authors for strawberries of the same varieties [32,33]. The ozone concentration significantly influenced the TPCs (Table 3). In general, in both strawberry varieties, the ozone application caused an increase in TPC content. However, in the SA variety, these increases were higher when using an ozone concentration of 0.3 ppm (Figure 5B). Onopiuk et al. [34] reported an increase in TPCs when treating strawberries with concentrations ranging from 0.3 to 1.2 ppm in a period of 60 and 180 min. These authors attribute this effect to the fact that ozone can activate ammoniacal phenylalanine lyase, an enzyme that participates in the synthesis of phenolic compounds. Other authors indicate that ozone can inhibit enzyme polyphenol oxidase and peroxidase, which are responsible for the oxidation of phenolic compounds from various fruits and vegetables [35].

The initial total anthocyanin content (TAC) was 4.09 ± 0.12 mg C3G/100 g FW and 4.42 ± 0.09 mg C3G/100 g FW for the CR and SA varieties, respectively (Figure 5C,D). The TAC values were in the range of concentrations reported by other authors for strawberries of the same varieties [36,37]. In the case of the TAC, the variety of strawberry and the storage days had a significant effect on this parameter (Table 3).

In this sense, the CR variety showed an increase in TAC during storage time (Figure 5C). Cordenunsi et al. [38] mention that the biosynthetic pathway of anthocyanins continues to be active after the strawberry harvest; furthermore, this process is not inhibited by storage at low temperatures. On the contrary, in the SA variety, the TAC decreased during the first three days of storage and after this time the concentration remained constant (Figure 5D).

Finally, for the antioxidant capacity, the strawberry variety had a significant effect on this parameter. The initial values of antioxidant capacity for CR and SA were 78.75 ± 6.98% and 49.35 ± 9.5%, respectively. The CR variety showed a higher antioxidant capacity compared to the SA variety (Figure 5E,F), which can be attributed to its higher concentration of TAC during storage.

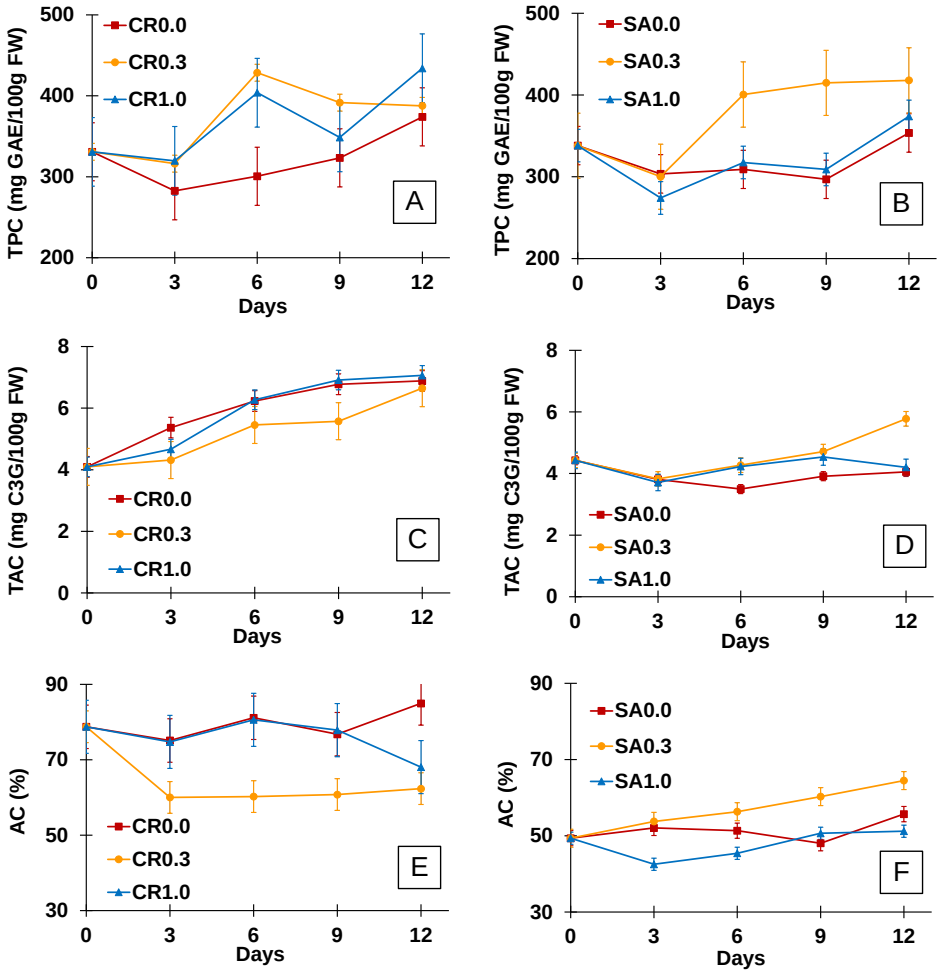

Figure 5. The effect of strawberry variety (CR: Camino Real and SA: San Andreas) and ozone concentration (0.0:0 ppm, 0.3:0.3 ppm, and 1.0:1 ppm) on total phenolic compounds: TPCs (**A**,**B**), total anthocyanin content: TAC (**C**,**D**), and antioxidant capacity: AC (**E**,**F**) of fruit during 12 days of storage (10 ± 0.2 °C). Error bars represent 95% confidence intervals.

Table 3. The results of three-way ANOVAs for bioactive compounds (TPC: total phenolic content and TAC: total anthocyanin content), microbiological analysis (AMB: aerobic mesophilic bacteria and M and Y: molds and yeasts), and antioxidant capacity (AC) of strawberries. The main effects and interactions of ozone concentration, strawberry variety, and storage time (significant results in bold).

	Bioactive Compounds						**Microbiological Analysis**			
	TPC		**TAC**		**AC**		**AMB**		**M and Y**	
	F	***p***	**F**	***p***	**F**	***p***	**F**	***p***	**F**	***p***
MAIN EFFECT										
Ozone concentration (A)	**4.819**	**0.015**	0.233	0.794	0.635	0.537	**13.774**	**0.000**	**6.621**	**0.005**
Strawberry variety (B)	0.447	0.509	**26.756**	**0.000**	**29.861**	**0.000**	**5.236**	**0.031**	0.925	0.346
Storage time (C)	0.762	0.558	**4.917**	**0.004**	0.185	0.944	0.554	0.651	1.918	0.154
INTERACTIONS										
A × B	2.471	0.102	1.371	0.269	**4.926**	**0.014**	**7.593**	**0.003**	0.954	0.399
A × C	0.088	0.999	0.425	0.896	0.139	0.997	2.070	0.095	**2.580**	**0.045**
B × C	0.025	0.999	**3.183**	**0.027**	0.393	0.812	1.861	0.163	0.232	0.873
A × B × C	0.037	1.000	0.612	0.761	0.094	0.999	1.526	0.212	0.638	0.699

3.3. Microbiological Properties

Figure 6 shows the effect of the ozone concentration and the strawberry variety on the inhibition of molds and yeasts in the fruit during storage. The initial loads of molds and yeasts for the CR variety were 2.10 ± 0.13 log10 cfu/mL and 2.73 ± 0.33 log10 cfu/mL, respectively. In the case of the SA variety, the initial loads of molds and yeasts were 2.17 ± 0.39 log10 cfu/mL and 2.47 ± 0.36 log10 cfu/mL, respectively. The ozone application had a significant effect on the loads of molds and yeasts (Table 3). In general, for both strawberry varieties, the samples that were not exposed to ozone (CR0.0 and SA0.0) presented a higher load of molds and yeasts with respect to the ozonated samples. Thus, the higher the concentration of this gas, the greater the inhibition of molds and yeasts in strawberries. For the CR variety, the samples exposed to 1 ppm ozone had a higher inhibition of molds and yeasts (2.47 and 2.79 log10 cfu/mL, respectively) during the first 8 days of storage. However, from this day onwards, microbial growth was observed (Figure 6A,C).

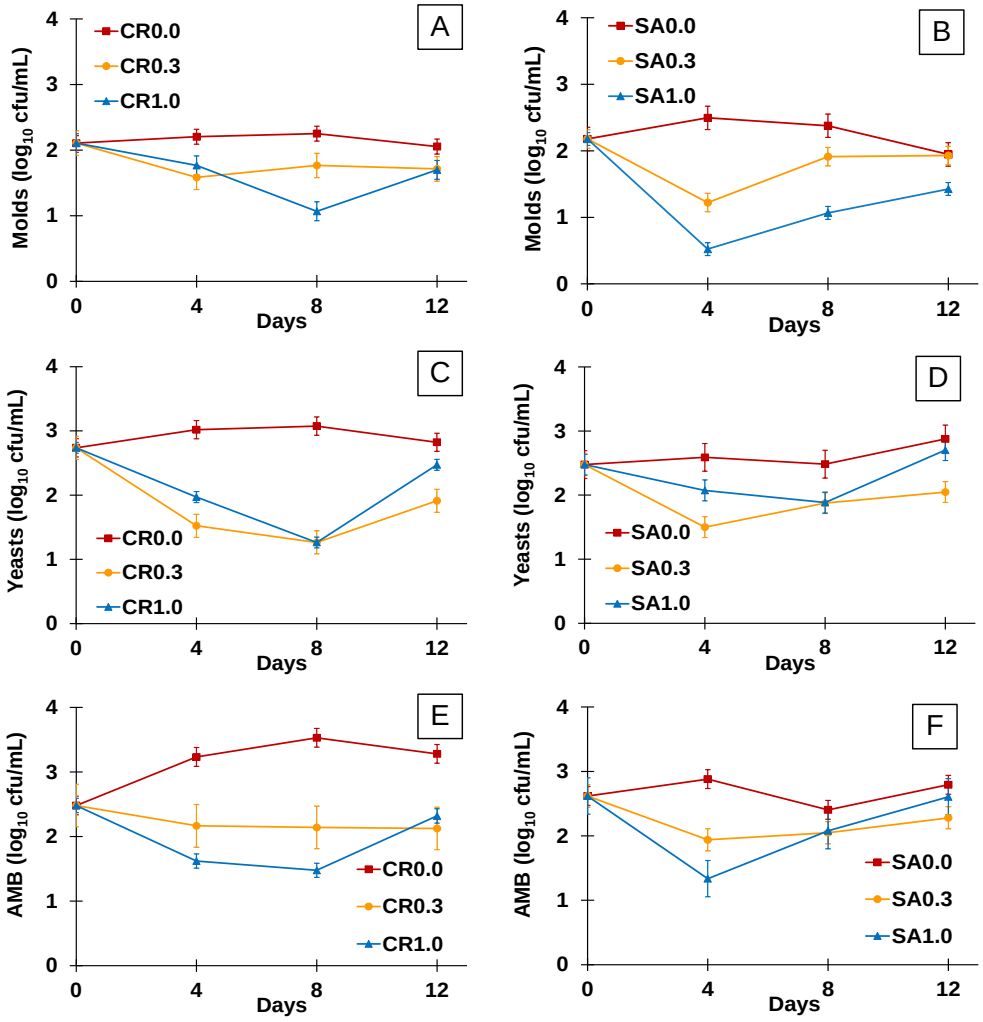

Figure 6. The effect of strawberry variety (CR: Camino Real and SA: San Andreas) and ozone concentration (0.0:0 ppm, 0.3:0.3 ppm, and 1.0:1 ppm) on molds (**A**,**B**), yeasts (**C**,**D**), and aerobic mesophiles bacteria (AMB) (**E**,**F**) of fruit during 12 days of storage. Error bars represent 95% confidence intervals.

In studying the inactivation processes of microorganisms assisted by ozone, van Boekel [39] reports that a certain population of microorganisms continues to be resistant to ozone exposure due to its adaptability. In this sense, and with the results obtained in our investigation, we assume that, in a certain population of microorganisms, ozone in the concentrations studied has a bacteriostatic effect. Finally, regarding the SA variety, the most

effective treatment for greater mold inhibition was 1 ppm ozone (Figure 6B). However, in the case of yeasts, a better reduction was obtained at 0.3 ppm (Figure 6D).

Regarding the aerobic mesophilic bacteria (AMB), the initial loads for the CR and SA variety were 2.48 ± 0.17 log10 cfu/mL and 2.61 ± 0.18 log10 cfu/mL, respectively. These reductions in AMB are consistent with what was reported by Maryam et al. [40] in inactivation processes with liquid ozone in strawberries. The ozone application and the strawberry variety had a significant effect on the AMB load (Table 3). In general, the samples without ozone treatment (CR0.0 and SA0.0) presented a higher load of AMB with respect to the ozonated samples (Figure 6E,F). For the CR variety, the ozone concentration of 1 ppm allowed a greater inhibition of AMB during the first 8 days of storage with respect to the rest of the treatments. In this sense, Khadre et al. [41] mention that, as the exposure time and ozone concentration increase, there is a greater interaction between ozone molecules and the cell surface of microorganisms. This fact causes damage to the cellular components, causing their partial or total inhibition. Regarding the SA variety, the samples treated with ozone (SA0.3 and SA1.0) had a greater inhibition of AMB during the first 4 days of storage. However, after this day they began to present an increase in microbial load.

4. Conclusions

Based on the results obtained in this research, we conclude that the effectiveness of gaseous ozone, as a postharvest treatment for strawberries, is modulated by the variety of the fruit and the ozone concentration used. When comparing both strawberry varieties, the CR variety was less susceptible to the negative effects of ozone compared to the SA variety. In general terms, for both varieties studied, the concentration of 0.3 ppm contributed to preserving most of the physicochemical, bioactive, and microbiological properties of the fruit. In addition, the application of ozone contributed to increasing the concentration of total phenolic compounds in the strawberry. As for perspectives for future research, it is important to perform sensory analysis on ozone-treated strawberries, characterize their present phenolic compounds, and further identify deteriorative microorganisms that may be tolerant to ozone application.

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