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Effectiveness of Ozonated Water for Preserving Quality and Extending Storability of Star Ruby Grapefruit

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Abstract: The aim of this study was to explore the impact of aqueous ozone technology on maintaining grapefruit flavor and freshness by minimizing the occurrence of postharvest deterioration. During the 2018 and 2019 seasons, Star Ruby grapefruit fruits were treated with 0.3 and 0.6 ppm aqueous ozone for 5 and 10 min after harvest at water temperatures of 5 °C and 15 °C, respectively. The fruits were stored for 40 days at 8 ± 1 °C with 85–90% relative humidity. The results revealed that all the ozonated water treatments reduced physiological weight loss, disease infection, and decay, as well as providing long-term protection to the fruits throughout storage. The best treatment for preserving the postharvest quality was 0.6 ppm ozonated water at 5 °C for 5 min, which successfully delayed ripening while concurrently preserving the TSS/acid ratios, total phenolics, and antioxidant activity. Overall, aqueous ozone treatment is a promising example of a treatment that is beginning to be utilized on a commercial scale. In accordance with the findings of this study, it can be deduced that aqueous ozone can be used to maintain fruit quality, reduce postharvest diseases, and extend storage life.

Keywords: Star Ruby grapefruits; cold storage; ozone; storability

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1. Introduction

Citrus fruits are the world's most widely produced fruits [1,2]. Grapefruit, which contains 91% water, is one of the most hydrating fruits. The most pigmented of the red grapefruit varieties, Star Ruby (*Citrus paradisi Macf.*), has a pleasing color, flavor, and aroma [3]. The majority of citrus fruit postharvest losses are caused by green *(Penicillium digitatum Sacc.)* and blue (*Penicillium italicum Wehmer*) mold [4,5]. Grapefruit's storage life is mostly limited by cold injury, weight loss, and fungal degradation. High doses of pesticides are employed in the postharvest treatment of fruits for the market, mainly to prevent fungal decay [6]. Chemical fungicides used to treat fungal infections in citrus fruits during postharvest storage are likely to be to be harmful to both human and environmental health. The water used to wash fruits and vegetables could include decaying bacteria and fungi. The use of chlorine-based compounds is a common method of minimizing microbial contamination. Recently, it was discovered that electrolyzed water (EW) made using sodium bicarbonate as an electrolyte reduced the population of *Penicillium* spp. in water, resulting in green mold deterioration in citrus fruits during storage [7]. Zamuner et al. [8] investigated the use of a cinnamaldehyde-based formulation as an alternative to sodium hypochlorite, which can damage the fruit peel, as a postharvest sanitizer for citrus fruit. As a result, innovative, environmentally friendly solutions to prevent rotting [9] and extend the life of citrus fruits after harvest are urgently needed [10]. To date, several non-chemical postharvest treatments for the control of *P. digitatum* and *P. italicum* have been investigated,

including irradiation, biocontrol agents, natural compounds, hot water treatment, and salts, in the hope of providing an alternative to the synthetic fungicides currently in use, which may have harmful effects on human health and the environment [11].

Ozone technology is one of the most environmentally friendly methods for decontaminating fresh fruits given that it leaves no toxic residue and keeps fruits safe and fresh [12,13]. The US Food and Drug Administration (USFDA) conferred often seen as secure (GRAS) status upon ozone in 2001 [14]. Ozone decontamination is a safe and efficient technique for minimizing fruit weight loss because it quickly decomposes into O_2 and does not collect dangerous secondary metabolites [5,15]. Antioxidant defense systems, both enzyme-based and non-enzymatic, scavenge reactive oxygen species (ROS) produced (directly or indirectly) by ozone exposure within cells, protecting cell structures and biomacromolecules from oxidative damage [16].

The short-term exposure level for ozone is limited to 0.3 ppm, according to the US Occupational Safety and Health Administration (US-OSHA) [17]. This is the highest concentration to which healthy persons can be exposed for 15 min without suffering discomfort or more severe symptoms. Ozone is also non-carcinogenic, which means it has no influence on the chemistry of healthy cells [18]. As a postharvest treatment, fruits can be treated using ozone in two forms: aqueous (ozone in water) [19] or gaseous (ozone in air) in storage chambers [5]. Ozone has 1.5 times the oxidizing potential of chlorine as a sanitizing agent and is significantly more effective at killing a larger range of microbes without leaving any toxicity [20].

The sweetness and overall acceptance of papaya treated with 2.5 ppm ozone has increased considerably. Furthermore, it demonstrated higher total soluble solids (25.0%), ascorbic acid content (12.4%), carotene content (19.6%), lycopene content (52.1%), and antioxidant activity (30.9%) than a control group, as well as less weight loss (11.5%) [15].

The effects of ozone on physicochemical qualities appear to be dosage-dependent [21]. As noted by Ong et al. [22], ozone fumigation at 5 ppm was able to lower the respiration rate and delay ripening in papaya compared to a control. To activate the antioxidative defense system and extend the shelf life of fruits, a sufficient amount of ozone is required. In this context, Artés-Hernández et al. [23] reported that ozone treatment increased antioxidant capacity, total phenol, and flavonoid concentration in table grapes. Furthermore, the strong oxidative characteristics of ozone influenced antioxidant and defense-related enzyme activity [24]. Ozone can be added to the environment, where the product is stored on a continuous or intermittent basis, or it can be dissolved in water to produce aqueous ozone for cleaning and sanitizing [25]. Ozone is effective against fungal postharvest disease and extends the shelf life of fruits by cleaning their surfaces [26]. The synergistic effect of both aqueous and gaseous ozone treatment, as mentioned by Karaca [27], can diminish *P. italicum* and *P. digitatum* mycelia growth on citrus while also slowing the aging process and boosting weight loss.

The major goal of this study was to examine the impact of postharvest treatments, such as aqueous ozone technology, on the storability and quality longevity of Star Ruby grapefruit during cold storage.

2. Materials and Methods

2.1. Fruit Material

The impact of aqueous ozone on the storability of Star Ruby grapefruit in cold storage was evaluated in this study over two seasons, 2018 and 2019. Grapefruit trees were grown in a private orchard on sandy clay soil and planted at 5 × 5 m with drip irrigation in EL-Nubaria region, Beheira Governorate (30.6667° N, 30.0667° E). Healthy fruits were picked in the last week of November during both seasons, when they were at their peak maturity stage (when more than two-thirds of the fruit surface show a yellow color and the total soluble solids/acid ratio is 6.5–7.5) [3]. The selected fruits were similar in size and appeared to be free of insect and pathogen damage; they were subsequently transported to the Horticulture Research Institute's fruit handling department's postharvest laboratory.

Freshly picked Star Ruby grapefruit were cleaned with wet foam in clean water, then dipped in 0.01 ppm chlorinated water for one minute and air-dried, and a rapid sorting was performed to detect flaws.

2.2. Preparation of Aqueous Ozone

The ozone was remedied by pumping oxygen through a discharge generator [19] (B6ATP, Euro Entech Co., Ltd., Mueang, Samut Prakan, Thailand) with a capacity of 2500 mg hG1. A closed-circuit ring device was formed when the generator was attached to a container filled with water (approximately 30 L; the ratio of the mass of the samples to the amount of ozonated water was roughly 80 g/30 L). The ozone generator produced between 0.3 and 0.6 ppm of dissolved oxygen when it was turned on. This attention was maintained throughout each session.

2.3. Treatment Applied and Storage Conditions

At the start of the experiment, 15 fruit samples were taken to determine the fruits' initial qualities. The remaining Star Ruby grapefruit were divided into six groups in a completely randomized design (3 replicates, 20 fruits each with every treatment containing 60 fruits), and then the fruits were immersed in ozonated water at varying concentrations (0.3 and 0.6 ppm) for 5 or 10 min at 5 °C or 15 °C, respectively. The following treatments were applied to fruits:

T1	0.3 ppm ozonated water for 5 min at 5 °C
T2	0.6 ppm ozonated water for 5 min at 5 °C
T3	0.3 ppm ozonated water for 10 min at 15 °C
T4	0.6 ppm ozonated water for 10 min at 15 °C
T5	Control dipping fruits with distilled water

The fruits were then dried before being packed in a single layer in 45 × 23 × 18 cm corrugated fibre board boxes, each with 20 fruits. Three boxes made up each treatment. The total number of boxes for all treatments was 15, and the storability of the fruits was evaluated after 40 days of storage at "8 ± 1 °C" with 85–90% RH.

2.4. Fruit Quality Attributes

2.4.1. Weight Loss

The weight loss was calculated by the accompanying equation [28]:

$$\text{Weight loss } \% = \frac{\text{Initial fruit weight} - \text{weight at sampling date}}{\text{Initial fruit weight}} \times 100$$

2.4.2. Disease Infection and Decay Percentage

Fruits were examined for the presence of fungi (*P digitatum* and *P italicum)* using a stereoscopic binocular microscope (6–50×). After examining the morphology of conidia and conidiophores, a compound microscope was used to validate the identification after evaluation [29].

Physiological disorders, fungal degradation, and physically detectable green calyx were all expressed as a percentage of the total fruits sampled. The incidence of physiological disorders was measured if the damage covered more than 25% of the rind surface.

All disease infections in fruits were calculated using the following formula [30]:

$$\text{Disease infection } \% = \frac{\text{No. of infected fruits}}{\text{No. of total fruits}} \times 100$$

Decay Percentage

A modified version of the methods described was used to compute the decay by Rokay, et al. [31]. The fruits were visually appraised and the weight of destroyed fruits was

recorded after 40 days of storage. The following equation was used to estimate all decaying fruits [28]:

$$\text{Decay } \% = \frac{\text{weight of decayed fruits}}{\text{Total weight of fruits at sampling date}} \times 100$$

2.4.3. Fruit Juice Percentage

The following formula was used to calculate the proportion of fruit juice:

$$\text{Fruit juice } (\%) = \frac{\text{Weight of juice } (\text{mg})}{\text{Weight of the whole fuits } (\text{g})} \times 100$$

2.4.4. Vitamin C (mg 100 g^{-1} Fresh Weight)

Ascorbic acid content in fruit juice was determined according the titration method described by Ranganna [32] using due solution with dichlorophenol and indophenol. The process involves converting 2,6-dichlorophenol indophenol dye to a colourless form in an alkaline solution of ascorbic acid for quantification. The reaction is for ascorbic acid, which is both quantitative and selective in solution, and occurs in the pH range of 1–3. To estimate the dye factor in the process that followed, the dye solution was first calibrated against standard ascorbic acid.

The phosphoric acid extract was titrated against the dye solution after diluting the sample with 3 percent metaphosphoric acid until a 15 s pink colour was produced. The dye factor was calculated using the following formula:

$$\text{Dye factor} = 0.5/\text{Titrate vol.}$$

Vitamin C as mg of 100 g^{-1} fresh weight ascorbic acid was estimated and calculated using the following equation:

$$\text{Vitamin C mg}/100\text{ g FW } = \frac{\text{Titrate vol.(mL of dye used)} \times \text{dye factor} \times \text{vol. made up} \times 100}{\text{Aliquot of sample taken for estimation} \times \text{vol. of sample}}$$

2.4.5. Total Soluble Solids (TSS %)

In 40 mL distilled water, 1 mL of fruit juice was dissolved. A Carl Zeiss hand refractometer was used to quantify total soluble solids in fruit juice. The findings were expressed as Brix according to A.O.A.C. [33]

2.4.6. Titratable Acidity (TA %)

To determine titratable acidity in fruit juice, 10 mL of sample was diluted with 100 mL of distilled water and 3–4 drops of phenolphthalein indicator were added. The citric acid in the fruit juice was titrated using 0.1 N NaOH according to A.O.A.C. [33].

The proportion of acid was determined using the formula:

$$\text{TA } \% = \frac{(\text{Titre vol.} \times \text{ Normality of NaOH } \times \text{ Vol. made up } \times \text{ Eq.wt. of acid}}{(\text{Aliquot of sample} \times \text{ Vol.of sample } \times 1000)} \times 100$$

2.4.7. Total Soluble Solids/Acid Ratio (%)

The TSS and titratable acidity results from fruit juice were used to compute this ratio.

2.4.8. Lycopene Assay

Ra, et al. [34] used a spectrophotometric method to determine the amount of lycopene in peel extract. Approximately 0.6 g of sample was added into a 50 mL centrifuge tube that contained HAE solvent (hexane, acetone, and ethanol in a 2:1:1 ratio). Samples were extracted for 15 to 30 min at 180 RPM. After shaking, 3 mL of deionized water were added to each tube and the samples were shaken for an additional 5 min. The tubes were then left at room temperature for 5 min to allow for phase separation. After separation, all extracted

lycopene was measured at 503 nm with the spectrophotometer using hexane as a blank. The total lycopene content was calculated using the following equation [35].

$$\text{Total lycopene content } = \frac{\text{A503} \times \text{MW} \times \text{DF} \times 100}{\varepsilon \times \text{L},}$$

where:

A503 is the absorbance of the hexane phase at 503 nm,
MW is the molecular weight of lycopene (536.9 g/mol),
DF is the dilution factor (mL/g),
ε is the molar extinction coefficient for lycopene in hexane (17.2 × 104 M/cm),
L is the light path (1 cm).

Calibrating curves were also created by diluting stock lycopene with methanol. The presence of all-*E* lycopene was determined by comparing the retention duration to that of the reference standard, and the quantity was determined using external standard calibration based on peak area. The carotene standard (1.25 g/mL) was added to the lycopene standard and utilized as an analytical process control. Total lycopene was calculated by adding the peak areas of all-*E* lycopene and the Z isomers and using the all-*E* lycopene standard curve [36].

2.4.9. Total Phenolic Compounds (mg g^{-1} FW)

Total Phenolics (TP) concentrations were assayed using Folin–Ciocalteu method, as described by Jayaprakash et al. [37]. In total, 8 mL distilled water, 0.1 mL extract, and 0.5 mL Folin–Ciocalteu reagent (1:1 with water) were combined in a 10 mL Eppendorf tube. After 1 min, 1.5 mL sodium carbonate (20 g per 100 mL) was added and thoroughly mixed. The reaction solution was then incubated in the dark for 2 h at room temperature before the absorbance was measured at 765 nm.

A standard curve of Gallic acid solution (25, 100, 300, 400, 500, 600, and 700 g/mL) was created and a calibration curve was developed by measuring the absorbance of Gallic acid concentrations and translating the data to mg g^{-1} FW gallic acid equivalent.

2.4.10. Antioxidant Activity (%)

The radical scavenging activity of DPPH (1,1-dihpenyl-2-picrylhydrazyl) was measured using the method described by Ao et al. [38]. The fruit peel was extracted using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) procedure, which makes use of methanol's DPPH free radical scavenging activity. A methanol extract (0.1 mL) was added to 0.9 mL of freshly produced DPPH methanol solution (0.1 mM). An equal amount of methanol was used as a control. After 30 min of incubation at room temperature in the dark, the absorbance (Abs) was measured with a spectrophotometer at 517 nm. The scavenging activity (%) was estimated using the formula below [39]:

$$\text{DPPH radical scavenging } (\%) = \left[\left(\frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}}\right) \times 100\right]$$

2.5. Statistical Analysis

Data were analyzed using one-way ANOVA, and statistically significant differences between treatment means ($\alpha \leq 0.05$) were determined using CoStat V6.4 program. The standard error (SE) of the mean is represented by vertical bars. Duncan's multiple range test was used as a post hoc test to determine whether there were any differences in treatment means between pairs.

3. Results

3.1. Weight Loss %

The data presented in Table 1 and Figure 1 show that there was a significant increase in weight loss percentage, of 11.25%, as the storage time increased. Furthermore, the results

reveal that dipping Star Ruby grapefruit in 0.6 ppm ozonated water at 5 °C for 5 min resulted in the smallest reduction in fruit weight loss (4.83% and 4.81%) after 40 days of cold storage in both seasons compared to the other treatments or the control. Furthermore, all of the treatments reduced the fruits' weight compared to the control. In both seasons, the untreated fruit lost 11.13% of their weight after 40 days of cold storage.

Table 1. Weight loss and decay percentage of Star Ruby grapefruits, stored at 8 ± 1 °C and 85–90% RH for 40 days during 2018 and 2019 seasons.

Treatments	Weight Loss (%) *		Decay (%) *	
	2018	2019	2018	2019
Control	0.00 ^{f}	0.00 ^{f}	0.00 ^{e}	0.00 ^{f}
0.3 ppm ozonated water at 5 °C for 5 min	11.25 ^{a}	11.13 ^{a}	10.21 ^{a}	10.36 ^{a}
0.6 ppm ozonated water at 5 °C for 5 min	0.00 ^{f}	0.00 ^{f}	0.00 ^{e}	0.00 ^{f}
0.3 ppm ozonated water at 15 °C for 10 min	5.60 ^{d}	5.63 ^{d}	5.23 ^{c}	5.26 ^{d}
0.6 ppm ozonated water at 15 °C for 10 min	0.00 ^{g}	0.00 ^{f}	0.00 ^{e}	0.00 ^{f}
	4.83 ^{e}	4.81 ^{e}	4.73 ^{d}	4.85 ^{e}
	0.00 ^{f}	0.00 ^{f}	0.00 ^{e}	0.00 ^{f}
	6.75 ^{b}	6.92 ^{b}	6.06 ^{b}	6.15 ^{c}
	0.00 ^{f}	0.00 ^{f}	0.00 ^{e}	0.00 ^{f}
	5.81 ^{c}	5.71 ^{c}	6.08 ^{b}	6.25 ^{b}

* means followed by the same letters are not significantly different according to Duncan's multiple range test at 0.05 level.

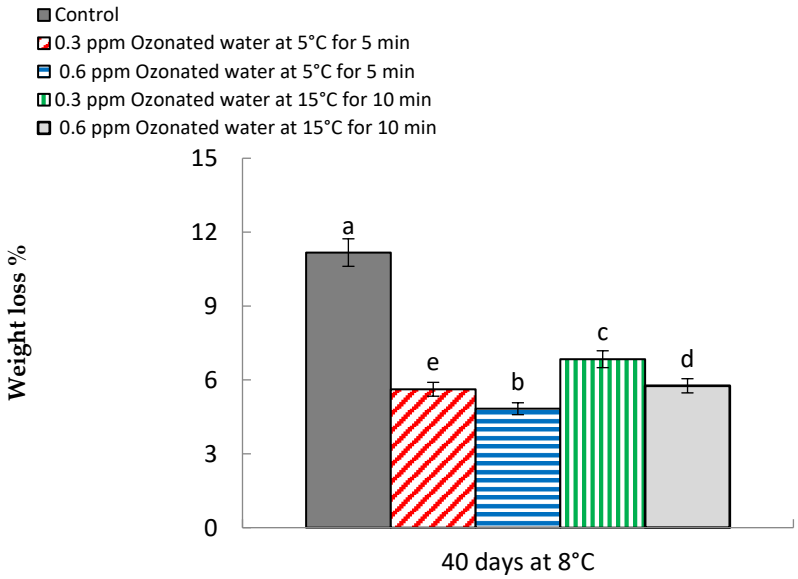

Figure 1. Weight loss percentage as a mean of Star Ruby grapefruits from two seasons, stored at 8 ± 1 °C and 85–90% RH for 40 days. Vertical bars indicate the standard error (SE) of the mean (n = 5). The superscript letters followed by the same letters are not significantly different according to Duncan's multiple range test at 0.05 level.

3.2. Decay Percentage

The data in Figures 2 and 3 show a reduction in disease infection after 40 days of cold storage at a lower water temperature (5 °C) and over a shorter period (5 min) in both seasons. In this regard, treatment with 0.6 ppm ozonated water at l5 °C for 5 min resulted in the greatest reduction in disease infection caused by *P. digitatum* and *P. italicum* (0.8% and 0.5%, respectively) as the mean of two seasons.

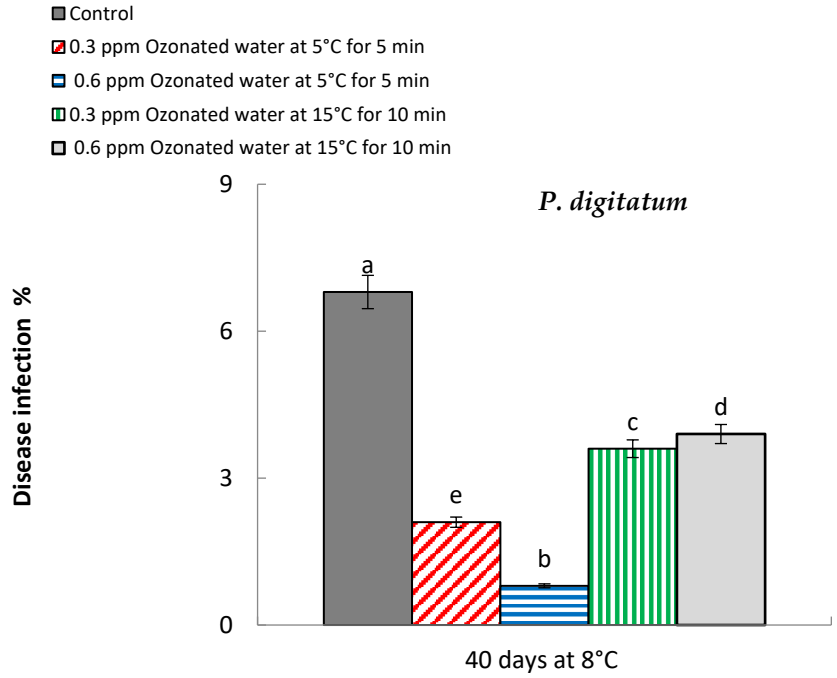

Figure 2. Disease infection percentage of *P. digitatum* as a mean of Star Ruby grapefruits from two seasons, stored at 8 ± 1 °C and 85–90% RH for 40 days. Vertical bars indicate the SE of the mean (n = 5). The superscript letters followed by the same letters are not significantly different according to Duncan's multiple range test at 0.05 level.

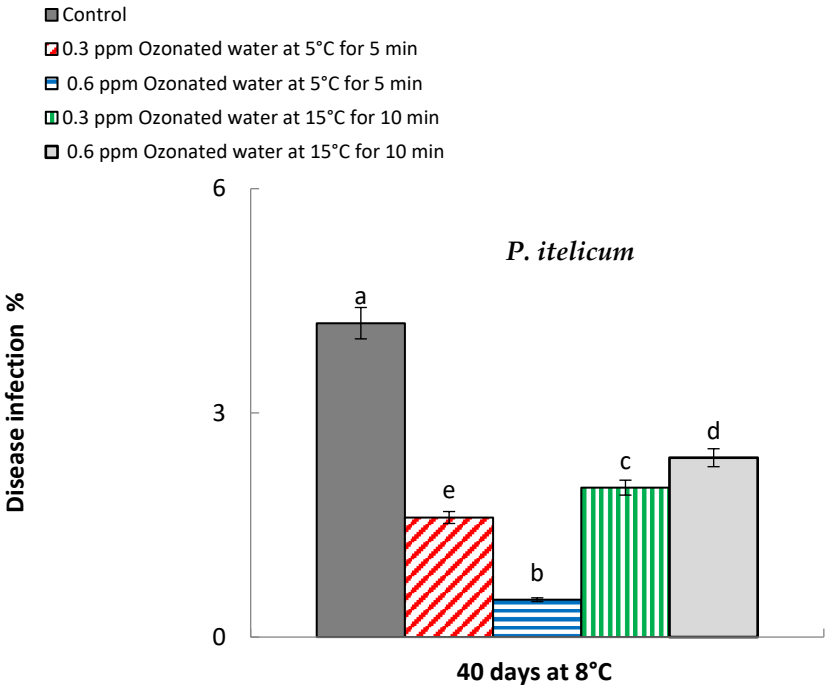

Figure 3. Disease infection percentage of *P. italicum* as a mean of Star Ruby grapefruits from two seasons, stored at 8 ± 1 °C and 85–90% RH for 40 days. Vertical bars indicate the SE of the mean (n = 10). The superscript letters followed by the same letters are not significantly different according to Duncan's multiple range test at 0.05 level.

Table 1 and Figures 2–4 show the impact of various applied treatments on the proportion of Star Ruby grapefruit that decayed during the two seasons. After the end of the cold storage, the decay percentage increased steadily. When compared to the untreated fruits, all the treatments led to a lower percentage of decaying fruits. During the 2018 and 2019 seasons, immersing Star Ruby grapefruit in 0.6 ppm ozonated water at 5 °C for 5 min resulted in the least significant percentage of decayed fruits (4.73% and 4.85%, respectively) after 40 days of cold storage. During the same period, in the 2018 and 2019 seasons, the incidence of fungal decay was highest in the control fruits (10.21% and 10.36%, respectively).

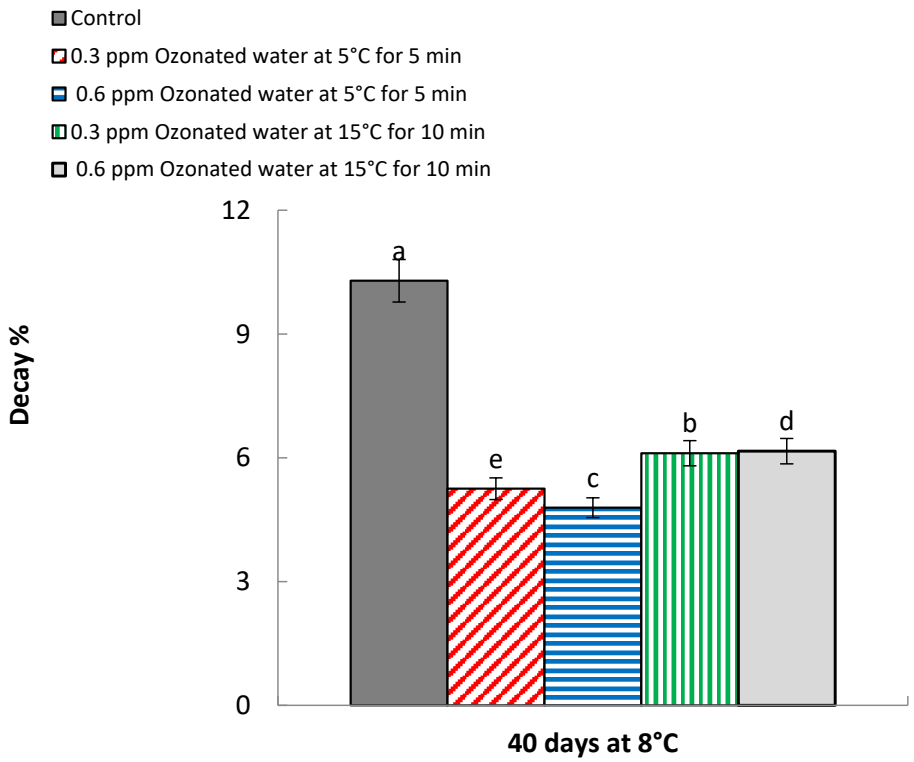

Figure 4. Decay percentage as a mean of Star Ruby grapefruits from two seasons, stored at 8 ± 1 °C and 85–90% RH for 40 days. Vertical bars indicate the SE of the mean (n = 10). The superscript letters followed by the same letters are not significantly different according to Duncan's multiple range test at 0.05 level.

3.3. Fruit Juice Content

All the treated fruits' juice percentages decreased when the storage period was increased from harvest to 40 days of cold storage, as shown in Table 2 and Figure 5. The obtained data showed significant differences among the treated fruits during the storage period. While dipping Star Ruby grapefruit in 0.6 ppm ozonated water at 5 °C for 5 min produced the highest juice content (45.76% and 40.17%) after 40 days of cold storage in 2018 and 2019 seasons, respectively, the minimum juice content (40.17% and 41.17%) was obtained from the untreated fruits (control) after 40 days of cold storage during the 2018 and 2019 seasons, respectively.

Table 2. Fruit juice content (%) and vitamin C (mg 100 g^{-1} FW) of Star Ruby grapefruits, stored at 8 ± 1 °C and 85–90% RH for 40 days during 2018 and 2019 seasons.

Treatments	Storage Period (days)	Fruit Juice (%) *		Vitamin C (mg 100 g^{-1} FW) *	
		2018	2019	2018	2019
Control	0	55.30 ^{a}	53.85 ^{a}	52.26 ^{a}	51.0 ^{a}
	40	40.17 ^{d}	41.17 ^{d}	45.26 ^{e}	44.26 ^{e}
0.3 ppm ozonated water at 5 °C for 5 min	0	55.30 ^{a}	53.85 ^{a}	52.26 ^{a}	51.0 ^{a}
	40	45.08 ^{c}	44.06 ^{c}	46.16 ^{c}	45.11 ^{c}
0.6 ppm ozonated water at 5 °C for 5 min	0	55.30 ^{a}	53.85 ^{a}	52.26 ^{a}	51.0 ^{a}
	40	45.76 ^{b}	44.66 ^{b}	46.54 ^{b}	45.53 ^{b}
0.3 ppm ozonated water at 15 °C for 10 min	0	55.30 ^{a}	53.85 ^{a}	52.26 ^{a}	51.0 ^{a}
	40	45.0 ^{c}	44.07 ^{c}	46.03 ^{cd}	45.0 ^{d}
0.6 ppm ozonated water at 15 °C for 10 min	0	55.30 ^{a}	53.85 ^{a}	52.26 ^{a}	51.0 ^{a}
	40	45.0 ^{c}	44.08 ^{c}	45.75 ^{d}	45.06 ^{c}

* means followed by the same letters are not significantly different according to Duncan's multiple range test at 0.05 level.

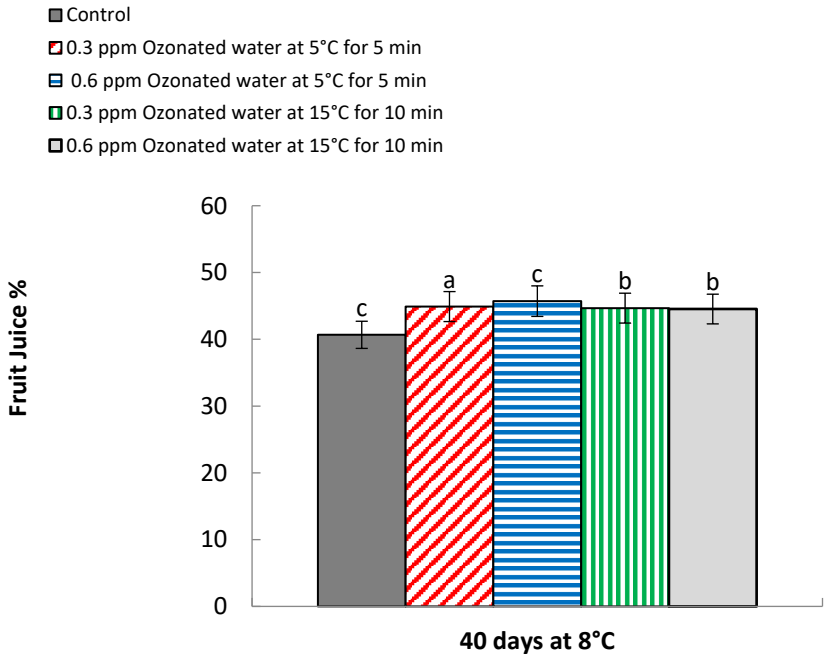

Figure 5. Fruit juice content (%) as a mean of Star Ruby grapefruits from two seasons, stored at 8 ± 1 °C and 85–90% RH for 40 days. Vertical bars indicate the SE of the mean (n = 10). The superscript letters followed by the same letters are not significantly different according to Duncan's multiple range test at 0.05 level.

3.4. Vitamin C (mg 100 g^{-1} FW)

The presence of vitamin C is among the most important quality parameters for fruits. The data encapsulated in Table 2 and Figure 6 evidence that the vitamin C decreased during storage period. When compared to the control, all the treatments preserved the loss of vitamin C in the Star Ruby grapefruit. In all the storage periods during the two seasons, the data showed significant values between treatments. In terms of the influence of different treatments on vitamin C concentration, the results showed that the fruits treated with 0.6 ppm ozonated water at 5 °C for 5 min after 40 days of cold storage had a higher vitamin C value (46.54 and 45.53 mg 100 g^{-1} FW) compared to other treatments or the control group during the 2018 and 2019 seasons, respectively. The untreated fruits had the lowest vitamin C values (45.26 and 44.26 mg 100 g^{-1} FW) after 40 days of cold storage in the 2018 and 2019 seasons, respectively.

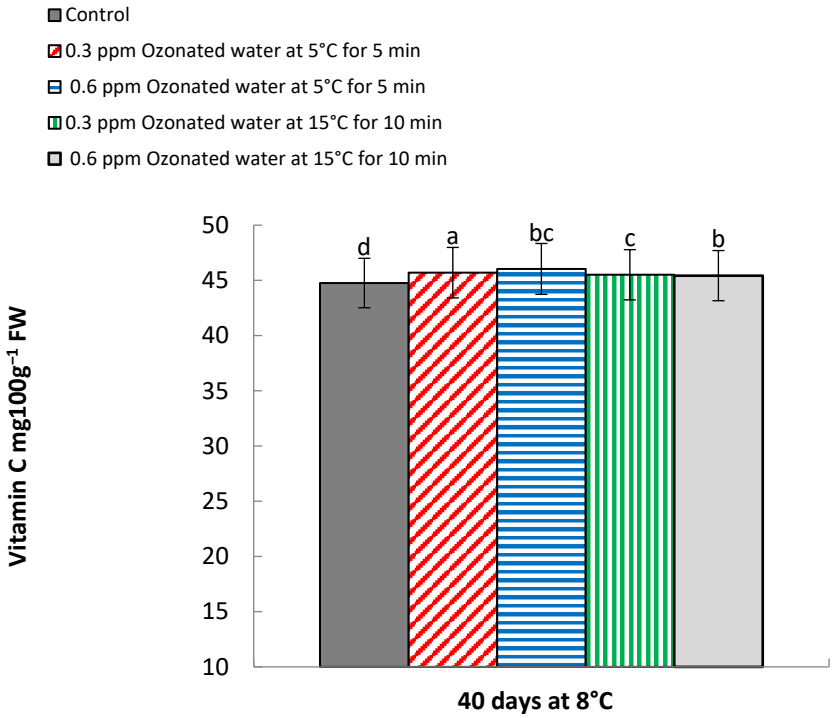

Figure 6. Vitamin C mg 100 g^{-1} FW a mean of Star Ruby grapefruits from two seasons, stored at 8 ± 1 °C and 85–90% RH for 40 days. Vertical bars indicate the SE of the mean (n = 10). The superscript letters followed by the same letters are not significantly different according to Duncan's multiple range test at 0.05 level.

3.5. Total Soluble Solid (TSS %)

Generally, the TSS and titratable acidity content of citrus fruits are crucial parameters for ascertaining quality. Although ozonated treatment slightly increases TSS and slightly decreases TA, the differences are small. In this study, ozonated treatment did not affect the TSS and TA of the fruit. In comparison to the control, all the treatments produced slightly greater TSS levels in fruit juice. The results in Table 3 and Figure 7 reveal that the treatment of 0.6 ppm ozonated water at 5 °C for 5 min produced a somewhat higher value of TSS in juice compared to the other treatments: 15.06% and 14.91% at the end of storage in both seasons, respectively. The untreated fruits also had lower TSS readings, which ranged between 14.64% and 14.24% during both seasons, respectively.

Table 3. Total soluble solid (TSS %) and titratable acidity (TA %) of Star Ruby grapefruits, stored at 8 ± 1 °C and 85–90% RH for 40 days during 2018 and 2019 seasons.

	Storage Period (Days)	TSS (%) *		Titratable Acidity (%) *	
		2018	2019	2018	2019
Control	0	13.98 ^{e}	13.84 ^{f}	2.05 ^{a}	1.95 ^{a}
	40	14.64 ^{d}	14.24 ^{e}	1.94 ^{b}	1.81 ^{b}
0.3 ppm ozonated water at 5 °C for 5 min	0	13.98 ^{e}	13.84 ^{f}	2.05 ^{a}	1.95 ^{a}
	40	14.73 ^{c}	14.33 ^{d}	1.84 ^{c}	1.73 ^{c}
0.6 ppm ozonated water at 5 °C for 5 min	0	13.98 ^{e}	13.84 ^{f}	2.05 ^{a}	1.95 ^{a}
	40	15.06 ^{a}	14.91 ^{a}	1.70 ^{d}	1.60 ^{e}
0.3 ppm ozonated water at 15 °C for 10 min	0	13.98 ^{e}	13.84 ^{f}	2.05 ^{a}	1.95 ^{a}
	40	14.77 ^{c}	14.46 ^{b}	1.83 ^{c}	1.73 ^{c}
0.6 ppm ozonated water at 15 °C for 10 min	0	13.98 ^{e}	13.84 ^{f}	2.05 ^{a}	1.95 ^{a}
	40	14.86 ^{b}	14.40 ^{c}	1.78 ^{c}	1.70 ^{d}

* means followed by the same letters are not significantly different according to Duncan's multiple range test at 0.05 level.

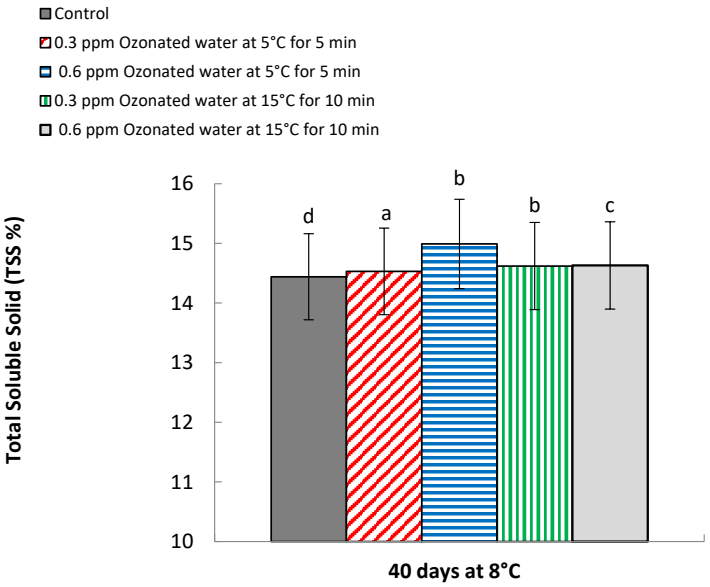

Figure 7. Total soluble solid (TSS %) mean values of Star Ruby grapefruits from two seasons, stored at 8 ± 1 °C and 85–90% RH for 40 days. Vertical bars indicate the SE of the mean (n = 10). The superscript letters followed by the same letters are not significantly different according to Duncan's multiple range test at 0.05 level.

3.6. Titratable Acidity (TA %)

The data in Table 3 and Figure 8 demonstrate that ozonated treatment did not affect the TA of the fruit. Generally, Star Ruby grapefruit treated with 0.6 ppm ozonated water at 5 °C for 5 min had the lowest TA percentages after 40 days of cold storage (1.70% and 1.60%) during both seasons, compared to the other treatments or the control. Furthermore, when compared to the other treatments, the fruit juice acidity was greater in the control fruits (1.94 % and 1.81 %), but it decreased with storage period due to the use of acids as respiratory substrates.

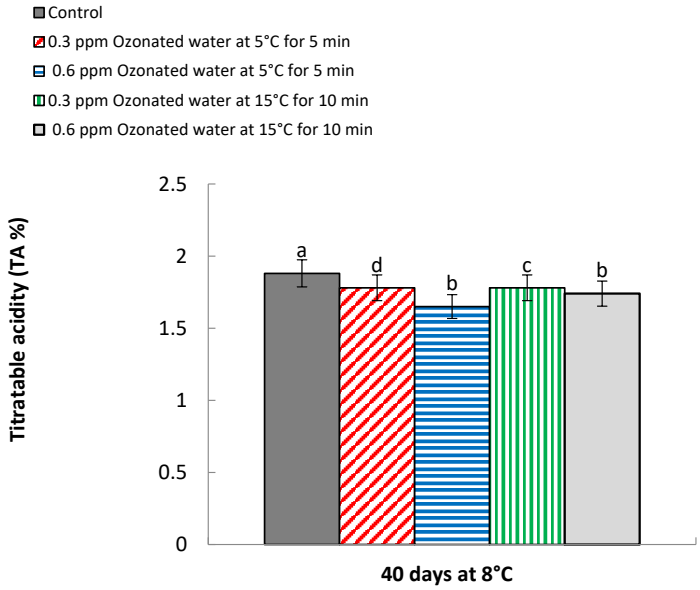

Figure 8. Titratable acidity (TA %) mean values of Star Ruby grapefruits from two seasons, stored at 8 ± 1 °C and 85–90% RH for 40 days. Vertical bars indicate the SE of the mean (n = 10). The superscript letters followed by the same letters are not significantly different according to Duncan's multiple range test at 0.05 level.

3.7. TSS/Acid Ratio (%)

In terms of the TSS/acid ratio, the data in Table 4 and Figure 9 reveal that as the storage period progressed from the harvest to the end of cold storage, the TSS/acid ratio increased. In other words, significant declining trends in juice titratable acidity percentage were observed. Furthermore, significant rising trends in TSS percentage were noted. After 40 days of cold storage in both seasons under consideration, all the treated fruits showed a somewhat higher TSS/acid ratio than the untreated fruits. The TSS/acid ratio in the control treatment was about 7.54% and 7.88% at the end of cold storage for the 2018 and 2019 seasons, respectively. Furthermore, during the two seasons, the fruits treated with 0.6 ppm ozonated water at 5 °C for 5 min had higher TSS/acid ratios, i.e., 8.85% and 9.31%, in 2018 and 2019 seasons, respectively.

Table 4. TSS/acid ratio (%) and total phenolic (mg g^{-1} FW) of Star Ruby grapefruits, stored at 8 ± 1 °C and 85–90% RH for 40 days during 2018 and 2019 seasons.

Treatments	Storage Period (Days)	TSS/acid Ratio (%) *		Total Phenolic (mg g^{-1} FW) *	
		2018	2019	2018	2019
Control	0	6.81 ^{e}	7.09 ^{e}	1093 ^{e}	1099 ^{f}
	40	7.54 ^{d}	7.88 ^{d}	1182 ^{d}	1172 ^{e}
0.3 ppm ozonated water at 5 °C for 5 min	0	6.81 ^{e}	7.09 ^{e}	1093 ^{e}	1099 ^{f}
	40	8.0 ^{c}	8.28 ^{c}	1186 ^{c}	1192 ^{d}
0.6 ppm ozonated water at 5 °C for 5 min	0	6.81 ^{e}	7.09 ^{e}	1093 ^{e}	1099 ^{f}
	40	8.85 ^{a}	9.31 ^{a}	1235 ^{a}	1245 ^{a}
0.3 ppm ozonated water at 15 °C for 10 min	0	6.81 ^{e}	7.09 ^{e}	1093 ^{e}	1099 ^{f}
	40	8.07 ^{c}	8.35 ^{c}	1191 ^{b}	1200 ^{b}
0.6 ppm ozonated water at 15 °C for 10 min	0	6.81 ^{e}	7.09 ^{e}	1093 ^{e}	1099 ^{f}
	40	8.34 ^{b}	8.47 ^{b}	1192 ^{b}	1196 ^{c}

* Means followed by the same letters are not significantly different according to Duncan's multiple range test at 0.05 level.

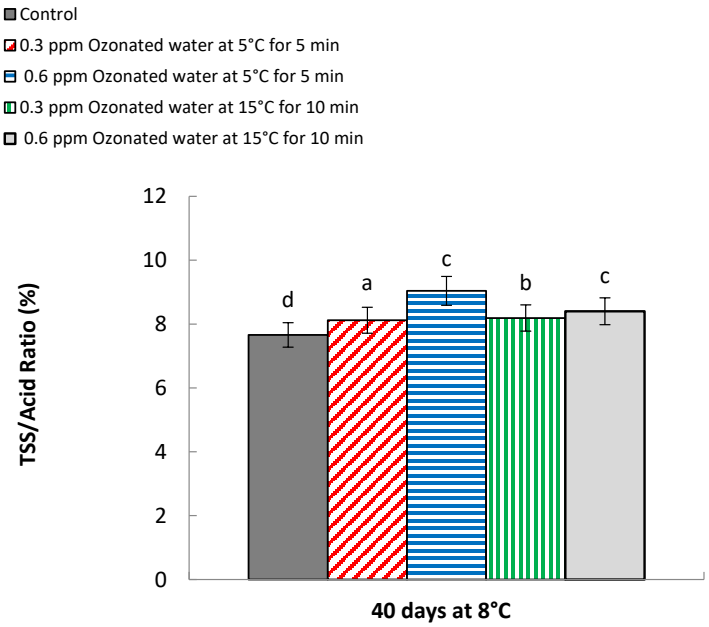

Figure 9. TSS/Acid Ratio % a mean of Star Ruby grapefruits from two seasons, stored at 8 ± 1 °C and 85–90% RH for 40 days. Vertical bars indicate the standard error (SE) of the mean (n = 10). The superscript letters followed by the same letters are not significantly different according to Duncan's multiple range test at 0.05 level.

3.8. Total Phenolic Contents (mg g^{-1} FW)

The total phenol content was increased by extending the storage duration, as shown in Table 4 and Figure 10. In comparison to the control sample, ozonated water treatments increased the total phenolics in the fruits. Furthermore, during the 2018 and 2019 seasons, the fruits dipped in 0.6 ppm ozonated water at 5 °C for 5 min had higher total phenolics, i.e., 1235 and 1245 mg g^{-1} FW, respectively. By contrast, the control fruits presented the lowest significant values of total phenolics (1182 and 1192 mg g^{-1} FW) during the two seasons of study.

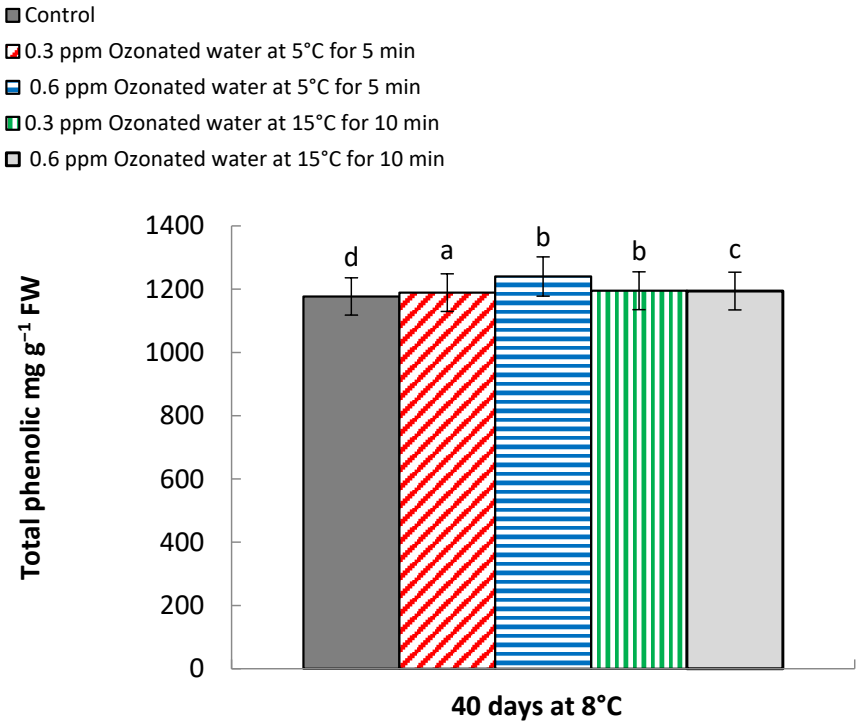

Figure 10. Total phenolic mg g^{-1} FW a mean of Star Ruby grapefruits from two seasons, stored at 8 ± 1 °C and 85–90% RH for 40 days. Vertical bars indicate the SE of the mean (n = 10). The superscript letters followed by the same letters are not significantly different according to Duncan's multiple range test at 0.05 level.

3.9. Lycopene Assay (mg 100 mL^{-1})

The results in Table 5 and Figure 11 show that during storage in both experimental seasons, the lycopene value was significantly increased by prolonging the storage period. Furthermore, when compared to the control, ozonated water significantly conserved the lycopene value. At the end of the storage period, dipping fruits in (0.6 ppm) ozonated water at 5 °C for 5 min kept the lycopene in the peel at 0.386 and 0.450 mg 100 mL^{-1} in the 2018 and 2019 seasons, respectively. By contrast, after 40 days of cold storage, the control fruits had the lowest lycopene levels in the peel, ranging between 0.326 and 0.360 mg 100 mL^{-1} in the 2018 and 2019 seasons, respectively.

Table 5. Lycopene assay and antioxidant activity (%) of Star Ruby grapefruits, stored at 8 ± 1 °C and 85–90% RH for 40 days during 2018 and 2019 seasons.

Treatments	Storage Period (Days)	Lycopene (mg 100 mL^{-1}) *		Antioxidant activity (%) *	
		2018	2019	2018	2019
Control	0	0.015 ^{e}	0.018 ^{f}	60.01 ^{f}	61.33 ^{c}
	40	0.326 ^{d}	0.360 ^{e}	61.15 ^{e}	62.0 ^{bc}
0.3 ppm ozonated water at 5 °C for 5 min	0	0.015 ^{e}	0.018 ^{f}	60.01 ^{f}	61.33 ^{c}
	40	0.383 ^{a}	0.420 ^{b}	62.01 ^{d}	62.13 ^{b}
0.6 ppm ozonated water at 5 °C for 5 min	0	0.015 ^{e}	0.018 ^{f}	60.01 ^{f}	61.33 ^{c}
	40	0.386 ^{a}	0.450 ^{a}	62.93 ^{a}	63.10 ^{a}
0.3 ppm ozonated water at 15 °C for 10 min	0	0.015 ^{e}	0.018 ^{f}	60.01 ^{f}	61.33 ^{c}
	40	0.363 ^{b}	0.383 ^{c}	62.10 ^{c}	62.23 ^{b}
0.6 ppm ozonated water at 15 °C for 10 min	0	0.015 ^{e}	0.018 ^{f}	60.01 ^{f}	61.33 ^{c}
	40	0.353 ^{c}	0.370 ^{d}	62.24 ^{b}	62.30 ^{b}

* means followed by the same letters are not significantly different according to Duncan's multiple range test at 0.05 level.

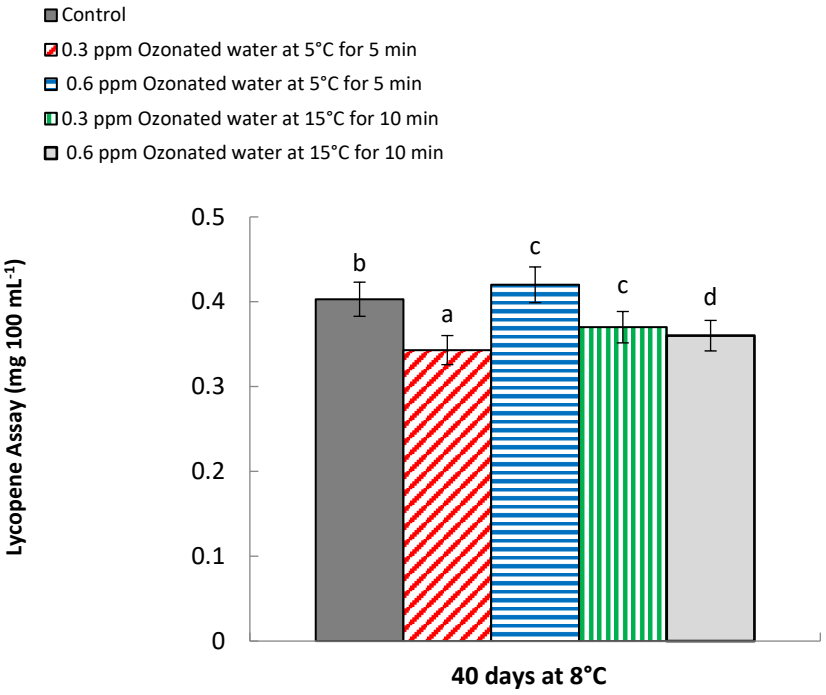

Figure 11. Lycopene assay (mg 100 mL^{-1}) mean of Star Ruby grapefruits from two seasons, stored at 8 ± 1 °C and 85–90% RH for 40 days. Vertical bars indicate the SE of the mean (n = 10). The superscript letters followed by the same letters are not significantly different according to Duncan's multiple range test at 0.05 level.

3.10. Antioxidant % (DPPH Radical Scavenging Assay)

The DPPH radical scavenging assay is commonly used to assess plant extract antioxidant activity. The effects of different ozonated treatments of Star Ruby grapefruits on the DPPH radical scavenging activity are presented in Table 5 and Figure 12. Among all the treatments applied after 40 days of cold storage in the 2018 and 2019 seasons, the fruits dipped in 0.6 ppm ozonated water demonstrated the highest percentage of inhibition (62.93% and 63.10%, respectively). The results also revealed that the untreated fruits significantly produced a lower percentage inhibition (61.50% and 62%) compared with all the treatments at the end of cold storage in the 2018 and 2019 seasons, respectively.

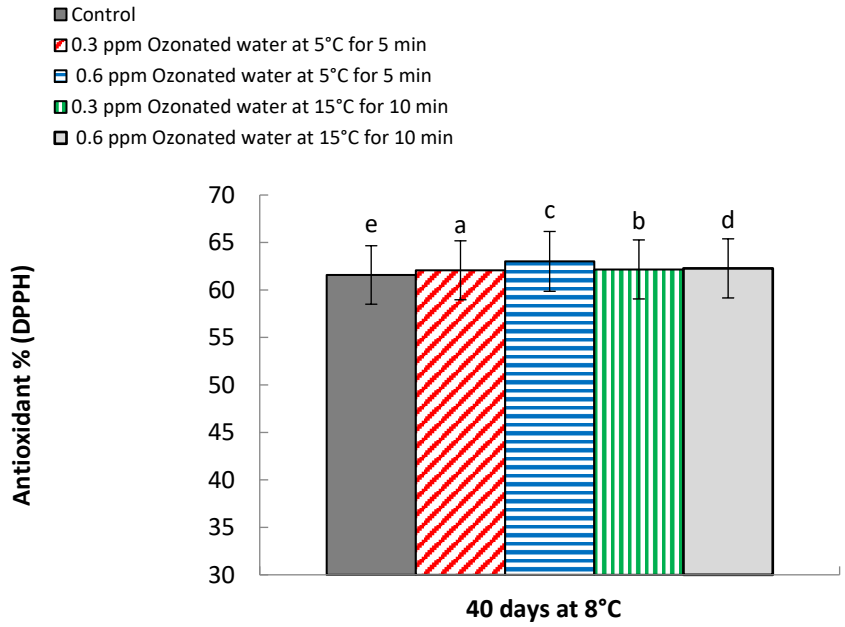

Figure 12. Antioxidant (%) (DPPH radical assay of fruit peel) a mean of Star Ruby grapefruits from two seasons, stored at 8 ± 1 °C and 85–90% RH for 40 days. Vertical bars indicate the SE of the mean (n = 10). The superscript letters followed by the same letters are not significantly different according to Duncan's multiple range test at 0.05 level.

4. Discussion

Both intrinsic and external aspects influence the efficacy of ozone. The kind and quality of fruit, as well as the microbial load of the strains, are extrinsic factors, whereas intrinsic factors include water quality (pH, organic matter, pressure, and temperature), air quality (air relative humidity RH], ozone concentration), and treatment time. Ozone solubility and stability improve as the temperature of an aqueous medium drops, increasing its availability in the medium and, as a result, improving its efficacy [40,41]. The breakdown rate of ozone increases as the temperature rises, making it less soluble and stable [42]. Lower water temperatures appear to increase the solubility of ozone in water, according to Palou et al. [43].

The data presented in Table 1 and Figure 1 reveal that, as expected, weight loss increased with storage time in all the samples. Furthermore, the results demonstrated that dipping Star Ruby grapefruit in 0.6 ppm ozonated water at 5 °C for 5 min resulted in the lowest amount of fruit weight loss after 40 days of cold storage in both seasons compared to the other treatments or the control. Weight loss contributes to a decline in citrus quality and increases susceptibility to fungal decay. The amount of water lost in the ozone-treated fruits was observed to be lower than in the control treatments (Figure 1).

Figures 2–4 show that after 40 days of cold storage at a lower water temperature (5 °C) and shorter period (5 min), disease infection was reduced. In this regard, during both seasons, treatment with 0.6 ppm ozonated water at 5 °C for 5 min resulted in the highest reduction in disease infection caused by *P. digitatum* and *P. italicum*. In general, the commercial ozone treatment of grapefruits can be utilized to minimize fungal rot. The main cause of fungal degradation in the control fruits was physiological disorders [44]. The presence of a localized brown-gray stain on the fruit surface was used to define physiological disorders in this case. During prolonged storage, this discoloration spread across the entire fruit surface, and secondary infections grew on the surface. Mycotoxigenic fungi were inactivated, and their mycotoxins were degraded using ozone [45]. Furthermore, using ozone to remove or reduce pesticide residues on fruits improves the flavor of most perishables by oxidizing pesticides and neutralizing the ammonia and ethylene gases created during ripening and decay [17,46]. The oxidative nature of ozone impairs microbial

cell protein synthesis and enzyme function, causing free radicals to break the cell membrane and cause microbial cell lysis [47]. Ozone has been shown to stimulate the development of phytoalexins in plants, which boost their resistance to degeneration and play a significant antioxidant role [48]. Furthermore, the same researchers discovered that a brief (20 min) exposure to ozone reduced the incidence of degradation in table grapes during cold storage, in addition to their subsequent shelf life. Ozone exhibited a sterilizing impact and induced resistance against the development of fruit rot [49]. By oxidizing cellular components such as sulfhydryl groups in amino acids in enzymes and oxidizing the cell membrane, ozone destroys microorganisms, extending shelf life and reducing shrinkage [50].

The juice content of all the treated fruits reduced when the storage period was extended to 40 days (Table 2 and Figure 5). The loss of juice content in the fruits during storage is indicated by the increase in weight loss. Our findings are consistent with those of Sakhale and Kapse [51].

Table 2 and Figure 6 show that, when compared to the control, all the ozonated water treatments maintained the loss of vitamin C in Star Ruby grapefruit. The preservation of ascorbic acid in the treated fruits could have been due to a decrease in ascorbic acid content respiration and oxidation, whereas the decrease in ascorbic acid in the control could have been due to an increase in respiration [52]. Furthermore, Horvitz and Cantalejo [53] discovered that ozone treatment increased the vitamin C content of strawberry fruits by promoting carbohydrate synthesis and activating antioxidative processes. The effect of temperature conditioning and/or storage time on ascorbic acid degradation could be explained by the direct oxidative destruction of ascorbinase activity or indirect degradation via polyphenol oxidase, cytochrome oxidase, and peroxidase activity [54].

TSS is an important quality attribute of most fruits. It represents the amount of soluble sugars and influences the flavor and marketability of fruit [55,56]. According to the data obtained in this study, the treatments had little effect on the TSS and titratable acidity of grapefruits. In this regard, all the treatments resulted in slightly greater TSS levels in fruit juice than the control. The decline in ethylene production is legitimately associated with the reduction in TSS %, which may have resulted in a decrease in sucrose synthesis due to the lower enzyme activity of sucrose–phosphate syntheses. Glucose and fructose (reducing sugars) levels in carrots treated with ozone at a concentration of 50 ± 10 nL L^{-1} rose slowly and linearly over a month of storage.

At the end of cold storage, it was obvious that the acidity content was rapidly decreasing. Commonly, the Star Ruby grapefruit fruits treated with 0.6 ppm ozonated water at 5 °C for 5 min had the least significant titratable acidity percentage compared to other treatments. The drop in acid concentration during fruit preservation was attributed to the use of acids in the fruit as a source of energy and respiration [57]. Furthermore, the inverse relationship between the TSS and the titratable acidity percentage was seen until the end of storage as a result of the drop in acidity caused by the metabolic process [58].

A fruit's flavor and nutritional quality are determined by TSS and titratable acidity. In comparison to the control, the postharvest administration of 0.6 ppm ozonated water at 5 °C for 5 min resulted in a greater TSS/acid ratio. In this respect, spraying Bidaneh Qermez grapes with 0.3 ppm ozone for 5, 10, and 15 min increased the amount of reducing sugars and the storage quality significantly compared to controls. According to a study conducted by Karaca and Velioglu [59], total acidity, total soluble carbohydrates, and flavonoid content were higher in mangos immersed in ozonized water. Physical characteristics such as color, flavor, and freshness diminished as the immersion time increased.

The fruits treated with ozonated water exhibited increased total phenolics in both seasons. Flavanone glucosides, p-hydroxybenzoic acids, and hydroxycinnamic acids are the main phenolic components in grapefruit and are all influenced by abiotic and biotic stress [60]. In general, the stimulation of the enzymes involved in the phenylpropanoid pathway, which rapidly increases their activity under ozone exposure as a defense mechanism operating in stress-affected cells, may explain why aqueous or gaseous ozone treatments enhance polyphenol content [61]. Sachadyn-Król et al. [62] observed an increase in total

phenolic content following ozone exposure owing to the stress caused by the gas's oxidizing characteristics and the radicals created during its decomposition, which were stabilized by phenolic compounds.

Ozonated water treatments significantly increased the lycopene value in the fruits compared to the control sample. The accumulation of lycopene in citrus fruit is of particular importance because it is a unique trait seen exclusively in a small number of species, such as grapefruit and pummelo. As a result, the presence of lycopene in Star Ruby and Flame grapefruits appears to be linked to a lower level of expression of the fruit-specific βLCY2 gene [63]. The amount of lycopene in a fruit depends on its maturity stage and the conditions in which it ripens. Furthermore, environmental changes may affect the synthesis of this pigment by inhibiting the fruit's metabolic activity, resulting in reduced ethylene production and fewer physiological changes [64].

The antioxidant properties of lycopene are likely linked to the activation of the antioxidant enzymatic system in citrus fruit flesh, according to Pan et al. [65]. The red parts of grapefruit's resistance to chilling damage could be due to an increase in antioxidant capacity caused by the tissue's high lycopene content [66].

Table 5 and Figure 12 show that the antioxidant activity in the peels of the Star Ruby grapefruits slightly increased as the storage period was extended during cold storage. The antioxidant activities in fruits increased during aging, and these increases could have been related to changes in the lipophilic antioxidant activity. Furthermore, antioxidant enzymes such as peroxidase, superoxide dismutase, and ascorbate peroxidase were vital in reducing the negative effects of oxidative stress during postharvest storage [67,68]. Alothman et al. [69] showed that ozone has a positive effect on total antioxidant capacity (TAC), which is commonly associated with an increase in total phenolic content (TPC) in minimally processed pineapple and banana after exposure to ozone.

The antioxidant system is activated as a result of the stress generated by a strong oxidizing agent, such as ozone, which improves the antioxidant status of horticulture crops. Immersion in ozonated water at 6 and 8 mg L^{-1} generates additional stress in grapefruit tissue, favoring the activation of enzymatic and nonenzymatic antioxidant stress mechanisms for detoxification. Gonzalez-Aguilar et al. [70] noted that fruits sterilized with ozonated water showed a high total polyphenol content and antioxidant capacity. As a result, ozonated water in doses 0.6 ppm at 5 °C for 5 min could be a viable choice for preserving fruit freshness for as long as possible.

5. Conclusions

In this study, the effect of aqueous ozone technology on the quality and freshness of Star Ruby grapefruits was explored. Fruits treated with 0.6 ppm ozonated water at lower water temperatures (5 °C) for 5 min after 40 days of cold storage showed physiologically desirable characteristics, such as delayed weight loss, disease infection, decay, and juice content maintenance. In comparison to all the treatments or the control, this treatment conserved TSS/acid ratio, total phenolics, lycopene, vitamin C, and antioxidant activity. As a result, aqueous ozone was validated as an effective new strategy for preserving the freshness of Star Ruby grapefruits after cold storage for as long as possible.

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