

Article

Short-Term Gaseous Ozone Exposure Influences Cucumber Quality During Postharvest Storage

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Abstract

Cucumber consumption has grown in the last decades, but its storage is challenging with alternative preservation means to require attention, including ozone. The present study examined the short-term application of gaseous ozone in cucumber fruit during storage. Fruits were exposed to ozone-enriched atmosphere (0, 0.2, and 1.5 ppm) for 2 or 6 h at 13 °C and 95% relative humidity, and then fruits were stored in clean air up to 7 days. The results showed that by maintaining low respiration rates, lower ozone concentrations (i.e., 0.2 ppm) and duration (2 h) were advantageous in sustaining cucumber quality-related traits. Furthermore, greener fruits, metabolic changes, and higher ascorbic acid content were the results of 0.2 ppm ozone exposure for 2 h or 6 h, but total phenols and malondialdehyde (MDA) levels were reduced. In contrast, higher ozone levels of 1.5 ppm for 6 h triggered fruit respiration rates, phenols, total soluble solids, and antioxidants alongside the raised ascorbic acid and MDA, resulting in less marketable products. Ozone treatments did not affect fruit aroma, appearance, and decay. Applying ozone for a short period of time may accelerate fruit metabolism and antioxidant responses to address the problem of oxidative stress.

Keywords: preservation; *Cucumis sativus*; oxidative stress; antioxidants; gaseous ozone

1. Introduction

Consumers are becoming increasingly interested in the nutritional value of foods and their potential health benefits as their knowledge of nutrition-related topics expands [1,2]. Consequently, researchers are searching more and more for new sources of naturally-occurring bioactive compounds that might contribute to human health and well-being [3,4]. Fresh vegetables, including cucumber (*Cucumis sativus* L.), are highly nutritious food, and greatly appreciated in our daily diet [5,6]. In the marketplace, cucumber ripeness is primarily assessed based on characteristics such as size, shape, and dark green skin color [7]. Cucumbers can be consumed raw in salads, pickled, or cooked. Global cucumber production was 94.7 million tons in 2022, indicating the cucumber cultivation as an important food sector and industrial factor [8]. Cyprus is a small country, with cucumber production of 7.6 thousand tons in 2022, contributing minimally to global production [8]. However, Cyprus is an island, quite isolated from the major food market scheme, with limited available fresh produce transportations due to their perishability and increased transport cost. Thus, fresh produce preservation strategies are more necessary to the island than the mainland.

Cucumber is composed predominantly of water and contains relatively low levels of fat, protein, and carbohydrates, while providing appreciable amounts of vitamins,



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minerals, dietary fiber, and organic acids [5]. Cucumbers are reported to exhibit several biocidal properties, including anticancer, antibacterial and antifungal [9], hypoglycemic and hypolipidemic [10], and antioxidant activities [6]. Cucumber has also traditionally been used to alleviate sunburn and for applications related to eye care [9].

Cucumber has a considerably short storage period, often restricted to less than 2 weeks due to the weight loss, decreased firmness, and discoloration [5,11]. It was reported that the retail losses were between 15 and 17% for cucumber [12]. Although chlorine treatment remains a common and widely used postharvest preservative mean nowadays, its effectiveness is questioned due to off-flavors, tasting alterations, aroma defects, and the possible generation of carcinogenic chloro-organic compounds [13,14]. In order to move closer to a total ban on the use of chlorine and bromine-based sanitizers in the coming years, European Union regulations (such as EEC 2092/91 and Biocides Directive 98/8/EC) impose stricter limitations on their use [15].

Consequently, potential safe but efficient sanitizing agents are of great importance to the fresh produce industry [16–18]. Several postharvest preservation methods have been investigated for cucumber, with promising effects, including 1-methylcyclopropene (1-MCP) [19], essential oils [5,20], UV-C [21], chitosan [20,22], propolis extracts [5], short-term hot water application [23], and modified atmosphere packaging [5]. Cucumber fruit is sensitive to chilling temperatures, particularly below 10 °C. However, cold acclimation has been shown to alter the proteomic profile of cucumber fruits and induce chilling tolerance [24]. A good substitute for the current postharvest preservation means palette is ozone (O₃) [25–27]. It is a non-thermal, environmentally friendly food preservation technique [28]. The US Food and Drug Administration (US-FDA) officially approved ozone as a food ingredient in 2003 after classifying it as GRAS (generally recognized as safe) in 1997 [13,29].

The benefits and applications of ozone for the preservation of fresh produce, including fruits and vegetables, have been extensively reviewed [15,26–28,30,31]. Research on ozone usage after harvest has been of great interest since the early 2000s [32–34], integrating the metabolic alterations that fresh produce undergo during storage with our own earlier research on the causes of ozone damage. Among its reported benefits, ozone action demonstrated antimicrobial properties and created features similar to vaccination against the growth of fungus in tomato, plum, and clementine [32,34]. Furthermore, it has been shown that ozone altered the protein profiles of tomatoes, strawberries, and pears [35–37], improved tomato, mango and cantaloupe quality [38–40], and increased fruit resistance by regulating several genes related to defense and signaling pathways [41] as well as the ascorbic acid–glutathione (ASA-GSH) cycle metabolism [42]. Ozone also appears to have positive effects on fresh produce's antioxidant qualities by activating processes that shield cellular structures from oxidative stress [1] as well as on phenylpropanoid metabolism [39]. Owing to its strong sanitizing activity, ozone has been widely investigated and applied for the preservation of fresh produce, particularly for controlling bacteria, fungi, and foodborne pathogens [26,43–45].

Previous studies on cucumber have investigated the continuous exposure to gaseous ozone at 0.1 µmol/mol (or 0.1 ppm) [46] at 14 °C for 14–17 days or 0.1 ppm ozone at 20 °C or 14 °C for 16 days. These treatments were associated with the maintenance of quality attributes, including reduced weight loss and better retention of green color [47]. Furthermore, short-term exposure to a relatively high ozone concentration of 10 ppm for 30 min, followed by storage at room temperature at 20 °C for 22 days, was reported to extend cucumber shelf life without the need for storing them at chilled conditions [48]. However, short-term ozone application in cucumber is less attended with gaps of knowledge in that direction. In previous reports, a 2 h exposure to ozone at concentrations ranging

from 0.05 to 0.2 ppm was effective in induced antimicrobial properties and signaling- and pathogenesis-related genes in tomato fruit [35]. Similarly, pepper fruit exposed to ozone for 1 and 3 h at 2 ppm at 25 °C had increased flavonoids [49]. Fresh products preservation is directly linked to the storage in low-temperature conditions. The research community had paid little attention to the biochemical mechanism of ozone's action that enhances its ability to preserve the quality of fresh horticulture products [50–52]. The purpose of the current study was to ascertain how short-term exposure to varying ozone levels (a considerably low concentration of 0.2 ppm and a moderate one of 1.5 ppm) for two or six hours affected the quality-related characteristics of cucumber fruits that were stored for seven days after harvest. The goal of this study was to increase our awareness and understanding of the benefits of ozone in short-term uses.

2. Materials and Methods

2.1. Fumigation System and Storage Conditions

Ozone treatments were carried out in chambers (internal volume: 15 L) placed within a walk-in controlled environment storage room. Ozone was produced via pure oxygen with an electric discharge device (BMOS OZ2000D, Bio-fresh Group Ltd., Stocksfield, UK). Ozone was delivered to each chamber through valved gap flow meters and distributed via a multi-channel PTFE[®] system. Ozone concentrations inside the chambers were measured with a photometric analyzer (UV-100, Bio-fresh Group Ltd., Stocksfield, UK), which sampled air from each chamber [53]. Due to the relevant small chamber volume, ozone treatment was not equipped with means of air movement.

Throughout the ozone application, chambers were kept at 13 °C and 95% relative humidity (RH), conditions previously shown to support ozone efficiency [26,35,38]. Temperature and RH were periodically recorded and logged using sensor devices. To preserve high RH levels, moist filter paper was positioned in each chamber.

2.2. Plant Material and Ozone Treatments

Cucumber (*Cucumis sativus* L. cv. PS-64) fruits (32 mm diameter; length of 18 cm, average fresh weight of 120 g) were collected from a local greenhouse farm (coordinates: 34°46'10.61" N; 33°11'22.08" E, 217 m; Limassol, Cyprus). The crop was cultivated using common cultivation practices in a clay sandy loam soil and was drip-irrigated and fertigated according to the crop's needs. Cucumber plants were trained on a string based on a vertical single pruning system (the main stem grew vertically, and lateral shoots were removed) and were grown for approximately 4.5 months. Crop cultivation was implemented during autumn/winter months in an unheated plastic greenhouse and the temperature ranged between 17 °C and 28 °C. Cucumbers were chosen at random based on their consistent size, appearance, and lack of physical defects. Therefore, 72 fruits were used across five treatments with 6 replicates × 2 pooled fruits (=60 fruits for the 7-day storage measurements), while the remaining 12 fruits were allocated at Day 0 baseline measurements, as described below. After their transfer to the laboratory, fruits were washed with chlorinated water (0.05% NaOCl), rinsed with drinkable and then with distilled water. Each treatment received a consistent distribution of fruits.

Ozone levels (a considerably low concentration of 0.2 ppm and a moderate one of 1.5 ppm) have been chosen based on previous experience of various ozone applications on tomato and pepper fruit with induced changes observed in fruit quality and secondary metabolites effects [26,35,38,49]. Cucumber fruits were exposed to air or ozone (0.2 or 1.5 µmol/mol (or ppm)) for 2 or 6 h (Figure 1). After ozone application, fruits were placed in plastic container (15 L capacity) and transferred to a storage room at 13 °C and 95% RH for 7 days storage in total. The experiment was conducted on six independent

biological replicates (each replicate consisting of two pooled fruits) per treatment. The containers were opened for aeration every other day to prevent any unintended rise in CO₂ and fall in O₂ during the fruit respiration process.

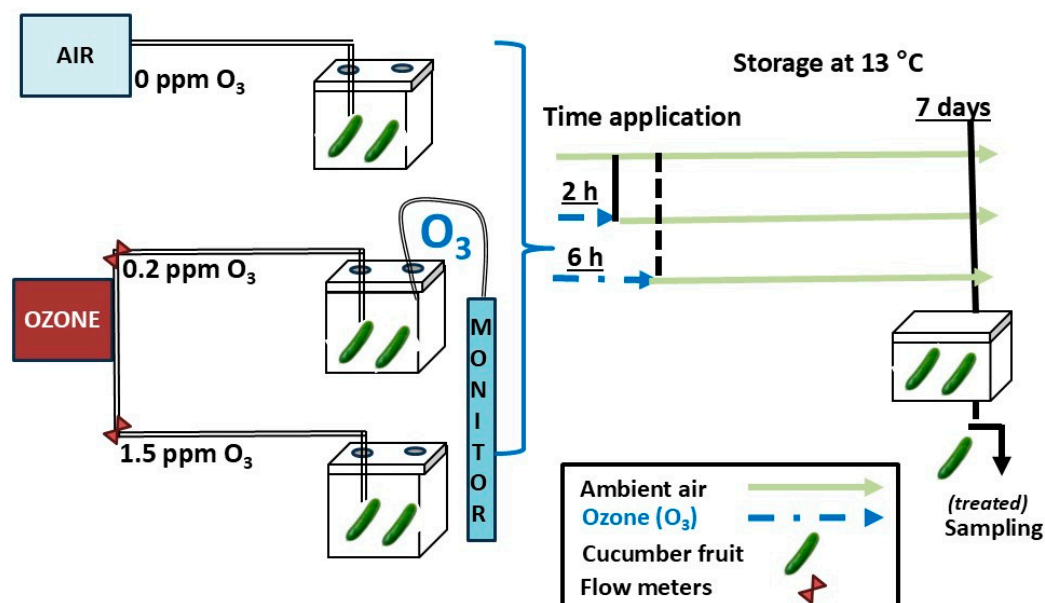


Figure 1. Experimental set up for ozone exposure (2 and 6 h) of cucumber fruits.

2.3. Impact on Fruits' Quality Attributes

2.3.1. Weight Loss and Respiration Rate

Cucumber fresh weight was initially recorded (Day 0) and then after 7 days of storage. The weight change (i.e., weight loss) was calculated and expressed as percentage value (%) of total weight loss.

Cucumber respiration rates were estimated before treatments (Day 0) and after storage (Day 7) as previously detailed by Xylia et al. [54]. Fruit was enclosed in a plastic container (5 L capacity) for 1 h, and the carbon dioxide (CO₂) produced was sucked from the container for 45 s by a dual gas analyzer (GCS 250 Analyzer, International Control Analyser Ltd., Kent, UK). The fresh weight and volume of each fruit were also measured. Fruit volume was determined by measuring the volume of water displaced when the fruit was fully submerged in a container filled with water. Results were calculated as mL of CO₂ generated per kilogram per hour (mL CO₂/kg × h).

2.3.2. Sensory Evaluation

Sensory evaluation of cucumber was performed by eight panelists (four males and four females; ages: 20–33 years old; introduced to cucumber's characteristics and storage conditions) that assessed the aroma, appearance, decay and marketability of the products on appropriate days [54]. Aroma evaluation was implemented using a 10-point scale (1 interval): 1: not cucumber and very unpleasant aroma, 3: not cucumber and slightly unpleasant aroma, 5: not cucumber but pleasant aroma, 8: less cucumber-like aroma and 10: intense cucumber-like aroma. Appearance (visual quality and color) was assessed using a 10-point scale (1 interval), 1: yellow color of 50%; 3: yellow-green; 5: light green; 8: green; 10: deep green. Marketability (indicating overall quality) was evaluated with the use of a scale of 1–10 (1 interval), where 1: not marketable quality (i.e., malformation, wounds, infection); 3: low marketability with malformation; 5: marketable with few defects, i.e., small size, decolorization, wilting (medium quality); 8: marketable (good quality); 10: marketable with no defects (extra quality). Decay was assessed using a 10-point scale

(1 interval): 1: 0–10% infection; 3: 21–30% infection; 5: 41–50% infection; 8: 71–80% infection; 10: 91–100% infection.

2.3.3. Fruit Color, Firmness, Total Soluble Solids, Titratable Acidity, and Ripening Index

The color on the surface of the fruits was determined with a colorimeter (Chroma meter CR400 Konica Minolta, Tokyo, Japan) by measuring the L^* , a^* , and b^* values (CIELAB uniform color space). Hue (h) value was determined in degrees ($^\circ$): $h = 180 + \tan^{-1}(b^*/a^*)$ [55]. The chroma value (C) was computed as $C = (a^{*2} + b^{*2})^{1/2}$, whereas color index (CI) was computed as $CI = (a^* \times 1000)/(L^* \times b^*)$ [55].

Fruit firmness was determined at two distinct sites on each fruit with a texture analyzer (TA.XT plus, Stable Micro Systems, Surrey, UK) equipped with a 3 mm diameter probe (penetration speed: 2 mm/s and penetration depth: 12 mm) [54]. The force required to break the fruit's pericarp was measured in Newtons (N). Drops of the extracted fruit juice were used to measure the total soluble solids (TSS) content by employing a digital pocket refractometer (Atago, Tokyo, Japan). Results of the TSS were presented as percentage (%). The titratable acidity (TA) of fruit juice was also determined by titrating it with 0.1 N NaOH using the previously described method [56], and the results were expressed as g of malic acid per L of juice. The ratio of TSS to TA (TSS/TA) was used to calculate the fruit ripening index.

2.3.4. Total Phenols, Antioxidant Activity, Ascorbic Acid

Total phenolics and the antioxidant capacity of cucumber were determined as previously described [57]. The Folin–Ciocalteu test was utilized to determine the total polyphenols in methanolic extracts, and the findings were reported as μg of gallic acid equivalents per g of fresh weight (μg GAE/g FW).

The following methods were used to measure the methanolic extracts' antioxidant activity: (i) the ferric reducing antioxidant power (FRAP) test; (ii) the 2,2-diphenyl-1-picrylhydrazyl (DPPH); and (iii) the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS). Both DPPH and FRAP assays were performed based on previously indicated methods [57]. The ABTS assay was conducted based on Wojdyło et al.'s [58] description. For all three antioxidant assays, results were presented as μg of trolox per g of fresh weight (μg Trolox/g FW).

Cucumber's ascorbic acid (AA) content was assayed by the 2,6-dichlorophenolindophenol titration method [56]. The results were reported as mg of AA per 100 g of fresh weight (mg/100 g).

2.3.5. Degree of Fruit Damage

The oxidative degradation of membrane lipids (lipid peroxidation) in cucumber was evaluated in terms of malondialdehyde (MDA) content by using the thiobarbituric acid reaction [59], while hydrogen peroxide (H_2O_2) content was determined based on the method described by Loreto and Velikova [60]. Both indicators reflected the fruit damage indices in the present research. Results were presented in nmol MDA and μmol H_2O_2 per g of fresh weight, respectively.

2.4. Statistical Analysis

The experimental setup for this study used a Completely Randomized Design (CRD), which involves six biological replicates for each measurement in each treatment. The statistical analysis was conducted using IBM SPSS version 25.0. The data was analyzed using one-way analysis of variance (ANOVA), and the means for each treatment were compared using Duncan's multiple range test at the significance level of $p = 0.05$. A principal component analysis (PCA) was carried out to detect the contribution of each variable to

the total diversity and classify the studied samples based on the tested parameters. The analysis was implemented with the statistical software MetaboAnalyst 6.0.

3. Results and Discussion

Fruit weight loss gradually increases during the storage period, while fruit water content is a key factor in determining fruit freshness and postharvest quality [61]. Figure 2 illustrates the impact of ozone enrichment on the weight loss and respiration rate of cucumber fruits. As shown in Figure 2A, no significant changes in weight loss were observed; the average weight loss remained at approximately 1.1% when fruits were subjected to low-level ozone treatments. This effect was similar to prior research in tomatoes exposed to continuous ozone treatment [38]. However, continuous ozone applications up to 17 days resulted in decreased cucumber weight loss [46]. This demonstrates that a suitable ozone dosage administration, due to the right concentration and duration, enabled cucumbers to keep their moisture during the storage period.

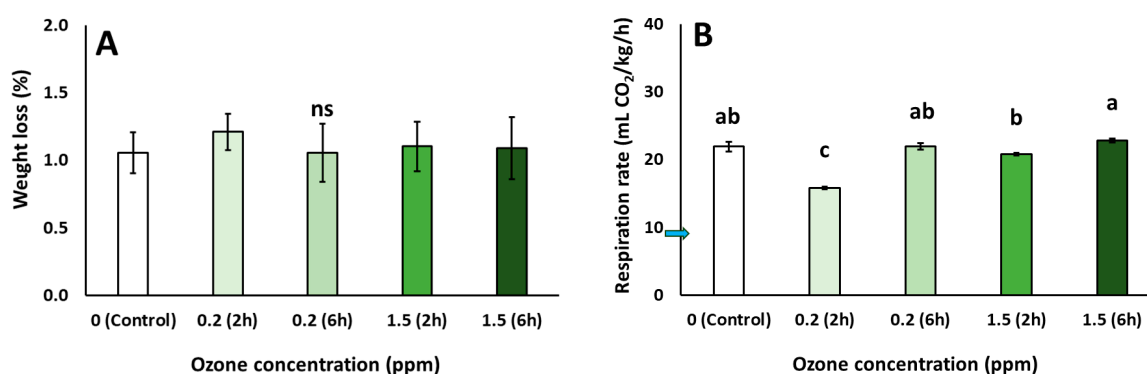


Figure 2. Impact of ozone enrichment on (A) weight loss and (B) respiration rate of cucumber fruits exposed to 0, 0.2, and 1.5 ppm ozone for 2 and 6 h and then stored for 7 days without ozone at 13 °C and RH 95%. Values represent the mean ($\pm$ standard error) of six independent replicates/treatment. The different lowercase letters indicate statistical differences ($p \leq 0.05$) among the treatments. Blue arrow indicates the values on day 0. ns: indicates no significance.

Lower respiration rate was observed on cucumber fruits exposed to ozone at 0.2 ppm-2 h whereas the highest respiration rate was evidenced in cucumbers treated with 1.5 ppm-6 h ozone, which was similar with the respiration rates of the control and 0.2 ppm-6 h ozone-treated fruits (Figure 2B). Those light differences in ozone-treated cucumber in respiration rates could be attributed to the non-climacteric fruit type, whereas in climacteric fruits, it was indicated that high ozone levels increased the cherry fruit respiration rates [62]. The treatment's efficacy may be impacted by the ozone dose. For example, when papaya fruit subjected to different ozone levels (0, 1.5, 2.5, 3.5, and 5.0 ppm), respiration rates decreased in fruits exposed in ≤ 3.5 ppm ozone [63]. The decreased respiration rates are linked to the decreased ripening and senescence process of fresh products [64].

Fruit color is often an important attribute that often reveals the fruit's age and degree of maturity [65]. Fruit lightness as measured by the L^* values, was increased in cucumbers exposed to 0.2 ppm-2 h compared with fruits exposed to 1.5 ppm-2 h ozone treatment (Figure 3A). Cucumbers exposed to 0.2 ppm-2 h ozone were greener (lower a^* value) and less yellow (higher b^* value), which mirrored the increased chroma value, compared to the non-ozonated fruits (Control) (Figure 3B,C,E). No differences were found in hue (Figure 3D) and color index (Figure 3F) values among the tested treatment, being in agreement with the results obtained in ozone-treated pomegranate [66]. Previous studies indicated that ozonated cucumbers maintained the green skin color [47], being in accordance with the current findings. Moreover, differences in color and chroma values were observed in cherry

fruits treated with ozone after the 14 days of exposure, whereas such changes were not evidenced with the shorter time of ozone exposure [62].

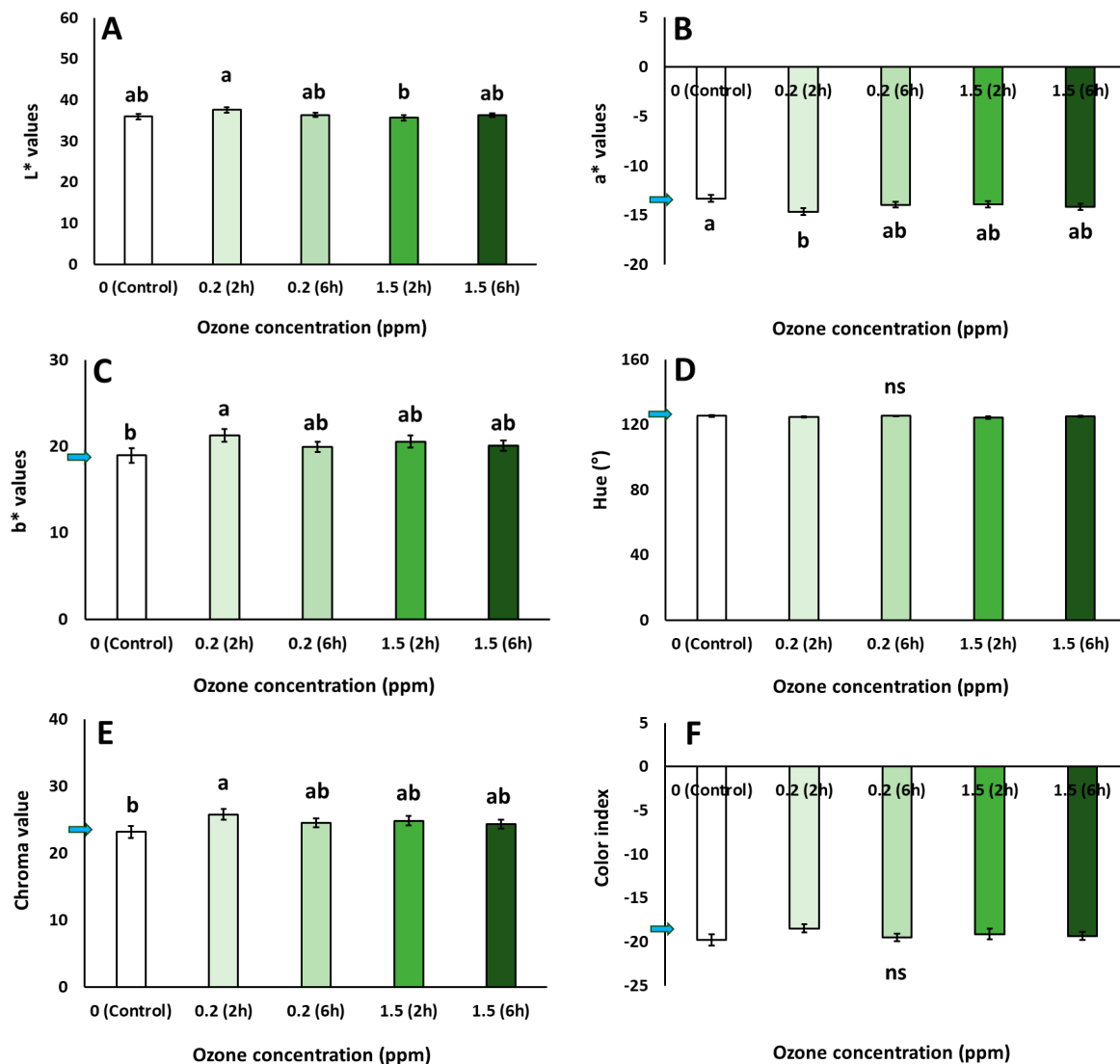


Figure 3. Impact of ozone enrichment on color index with (A) L^* value, (B) a^* value, (C) b^* value, (D) hue values, (E) chroma value, and (F) color index of cucumber fruits exposed to 0, 0.2, and 1.5 ppm ozone for 2 and 6 h and then stored for 7 days without ozone at 13 °C and RH 95%. Values represent the mean ($\pm$ standard error) of six independent replicates/treatment. The different lowercase letters indicate statistical differences ($p \leq 0.05$) among the treatments. Blue arrow indicates the values on day 0. ns: indicates no significance.

Fruit skin color vibrancy is expressed by the chroma value, and a rise in this value indicates an improvement in color purity [66]. The increased chroma values observed in cucumber fruits when subjected to 0.2 ppm-2 h ozone treatment could not be identified via macroscopical (Figure 4) and organoleptic assessment (Figure S1B). This highlights the importance of using organoleptic evaluation and acceptability tests by the consumers in addition to the laboratory measures. Notably, consumers are the final decisions makers, and their valuable justification needs always to be considered. Fruit marketability was slightly decreased in cucumbers exposed to 1.5 ppm-6 h ozone treatment compared to the control treatment (Figure S1C). Fruits exposed to different ozone treatments had the same aroma scores (Figure S1A), without any decay symptoms (Figure S1D). Similarly, pomegranate fruits exposed to ozone had similar appearance and taste values with the

untreated fruits [66]. It is noteworthy to mention that after tomatoes were exposed to continuous ozone for six days, a panel review found that the ozonated fruits were sweeter than the control [38]. During storage, fruit deterioration increases, initially with relevant slow speed and then accelerating later. Fruit decay incidence was decreased after a continuous ozone application in cucumber for 14 days [47]. The storage period in the present study was 7 days, which might justify the absence of any decay symptoms among the tested treatments.

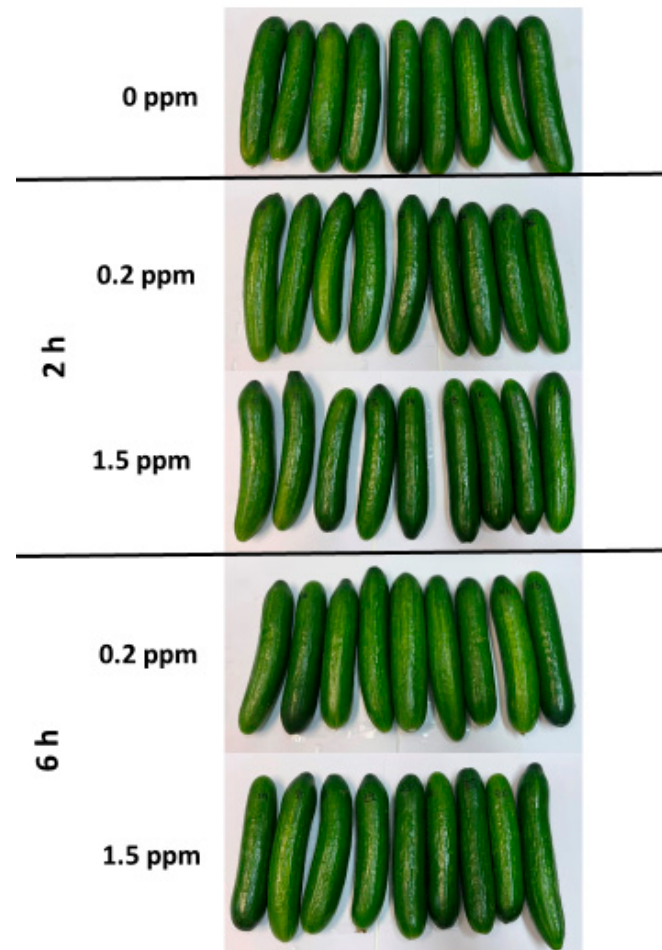


Figure 4. Impact of ozone enrichment on cucumber fruits exposed to 0, 0.2, and 1.5 ppm ozone for 2 and 6 h and then stored for 7 days without ozone at 13 °C and RH 95%.

Fruit texture, often referred and measured by the fruit firmness, is a crucial factor of the quality and storage potential of fresh products as it shows structural integrity, retained water, and the aging process over time [61]. Fruit firmness decreased at the 1.5 ppm-6 h ozone-treated cucumbers compared to the 1.5 ppm-2 h ozone-treated fruits at the end of storage (day 7); however, no changes were found with the other ozone treatments and/or control (Figure 5A). In previous studies, when cucumber fruits were continuously exposed to 0.1 ppm of ozone for 14 days, fruit firmness was maintained to similar levels as the control fruits [47], being in line with the present results. In another study, it was reported that blackberry firmness was higher in ozonated fruits compared to the relevant control (untreated) fruits [67]. Previous reports indicated the correlation between tomatoes' shelf life, fruit firmness, and malate concentration [68]. This is in accordance with the present findings, as cucumber firmness was correlated with malic acid content ($y = 0.0935 x$; $R^2 = 0.9814$). It was suggested that malate measures can act as a good biomarker for genotypes selection

with improved quality attributes [61]. Interestingly, treatment with ozone may alter the production of suberin, changing the firmness of cantaloupe [69].

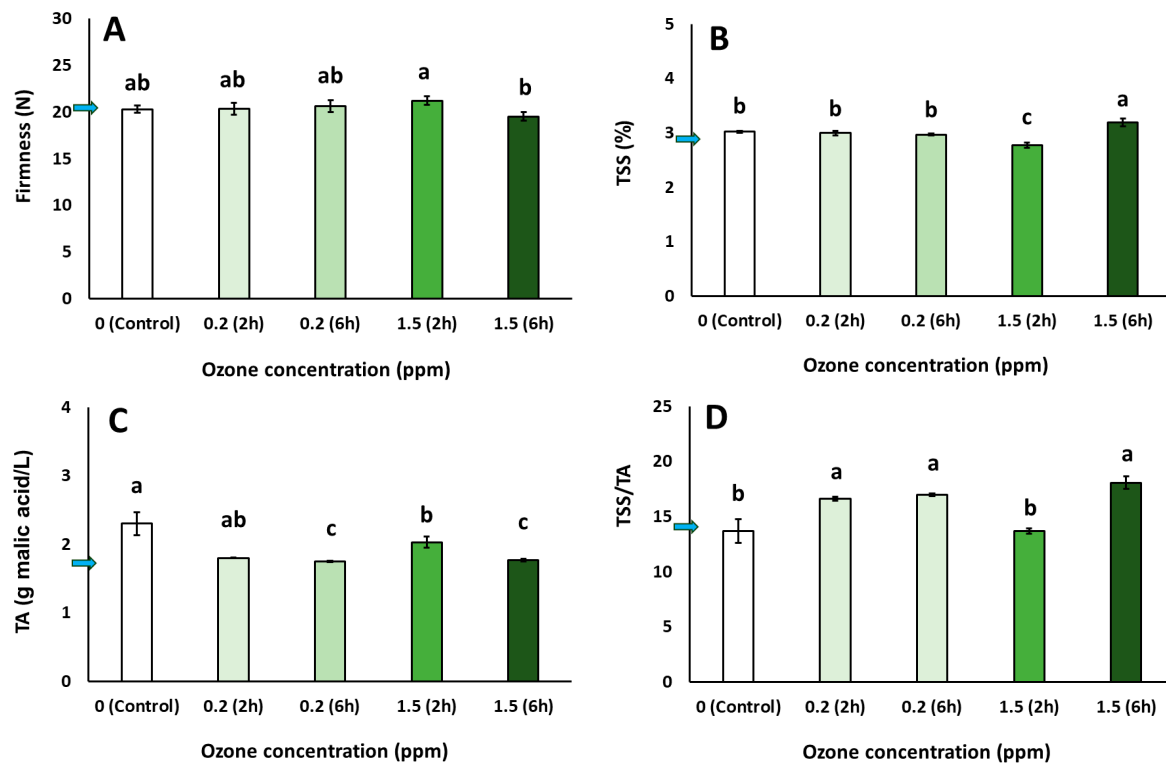


Figure 5. Impact of ozone enrichment on (A) firmness, (B) total soluble solids (TSS), (C) titratable acidity (TA), and (D) ripening index (TSS/TA ratio) of cucumber fruits exposed to 0, 0.2, and 1.5 ppm ozone for 2 and 6 h and then stored for 7 days without ozone at 13 °C and RH 95%. Values represent the mean ($\pm$ standard error) of six independent replicates/treatment. The different lowercase letters indicate statistical differences ($p \leq 0.05$) among the treatments. Blue arrow indicates the values on day 0.

Total soluble solid content, which is frequently used to assess sugar level, exhibits varying behavior after harvest, mostly based on the species and storage parameters [61]. The highest total soluble solids were found in 1.5 ppm-6 h ozone-treated fruits (Figure 5B), where the same fruits (including the 0.2 ppm-6 h ozone treatment) revealed the lowest titratable acidity (Figure 5C). Consequently, increased TSS/TA ratio, an indirect maturity index, was found in 0.2 ppm-2 h, 0.2 ppm-6 h, and 1.5 ppm-6 h ozone-treated fruits (Figure 5D). The TSS/TA ratio is an indirect maturity index because changes in soluble solids and titratable acidity may also arise from water status, respiration, carbohydrate metabolism, cultivar, and environmental conditions, rather than ripening alone [70]. Similar to the present results, ozone treatment delayed TA loss compared to control in pomegranate [66]. However, other studies showed that TSS was unchanged in fruits exposed to ozone, as it was in the case of apples [71], kiwifruit [1], and tomatoes [38]. These variations can be related to the ozone levels and storage conditions as well as to the ripening metabolism of individual fresh products, considering climacteric (i.e., apple, kiwifruit, tomato) and non-climacteric (i.e., cucumber) fruits.

The greatest content of total phenols was shown to be in 1.5 ppm-6 h ozone-treated fruits, whereas decreased levels were found in low ozone (0.2 ppm)-treated fruits compared to the control (Figure 6A). Similarly, total phenolic content maintained or increased in ozonated cherry fruit as moderate ozone levels helped to slow down the phenols degradation [62]. Morais et al. [72] reported greater total phenols levels in ozonated (1 g/h for

20 min) strawberries compared to the untreated control, whereas Sachadyn-Król et al. [49] reported increased total phenols in ozonated (2 ppm for 1 h) peppers and storage at 25 °C for 20 days. Total phenolics in kiwifruit rose after 15–30 min of exposure to ozone at relevant elevated levels of 100 ppm [1], suggesting that total phenol content may be raised at relevant high ozone doses. It is notable that the relevant high doses in the current study were 9 ppm × h, whereas the ozone doses in the Piechowiak et al. [1] study were 25–50 ppm × h. In another study where apples were subjected to ozone at 2.5 ppm for 2 h for one month, total phenols were decreased, while higher ozone levels of 5.0 ppm for 2 h caused black spots symptoms on the fruit surface [71]. The phenolic compounds levels can be increased, decreased, or remain unchanged based on the ozone concentration, time duration, fresh products, and storage conditions [30].

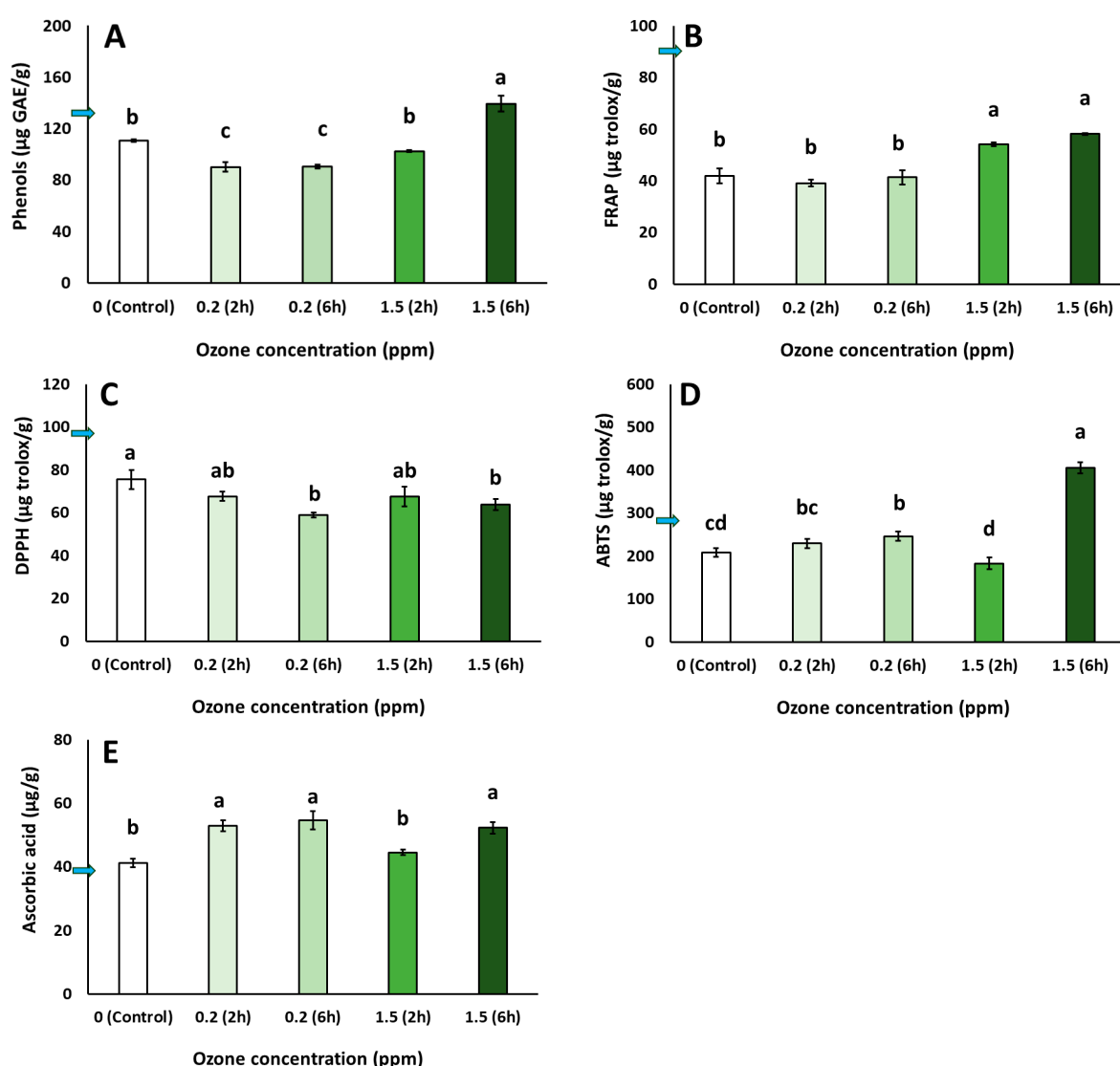


Figure 6. Impact of ozone enrichment on (A) total phenols, antioxidant capacity with (B) FRAP, (C) DPPH, (D) ABTS, and (E) ascorbic acid (AA) of cucumber fruits exposed to 0, 0.2, and 1.5 ppm ozone for 2 and 6 h and then stored for 7 days without ozone at 13 °C and RH 95%. Values represent the mean ($\pm$ standard error) of six independent replicates/treatment. The different lowercase letters indicate statistical differences ($p \leq 0.05$) among the treatments. Blue arrow indicates the values on day 0.

Antioxidant capacity as assayed by FRAP revealed increased values in high levels of ozone (i.e., 1.5 ppm for 2 h or 6 h) (Figure 6B). Similarly, increased ABTS was found in

1.5 ppm-6 h treated fruits (Figure 6D). The DPPH values decreased in fruits exposed in 0.2 ppm-6 h and 1.5 ppm-6 h ozone treatment compared to the control (Figure 6C). Previous reports indicated that ozone treatment could maintain higher levels of antioxidants [62,63] as it was found for the FRAP and ABTS measures at the higher ozone doses applied. Similar findings were also observed when cucumber and leeks were washed with ozonated water whereas reverse results were evidenced in bell pepper, parsley, cabbage, and rocket, highlighting the different responses of various fresh produce in ozone applications [73].

Vitamin C, as shown by the ascorbic acid level, is a significant biochemical indication of the antioxidant activity and nutritional value of fresh products. Ascorbic acid content was increased in fruits exposed to 0.2 ppm-2 h, 0.2 ppm-6 h, and 1.5 ppm-6 h ozone applications compared with the control and 1.5 ppm-2 h ozone treatments (Figure 6E). The increased levels of AA in ozonated cucumber indicate that the right ozone levels may postpone the AA degradation. Similarly, Horvitz et al. [67] reported increased AA content in blackberries after ozone exposure, while Morais et al. [72] reported greater AA levels in ozone-treated (1 g/h for 20 min) strawberries compared to the untreated control. Moreover, increased AA levels were obtained in ozone-treated kiwifruit after ozone exposure (79.44 ppm for 1 h) once a day for 7 days [74]. It is noteworthy that greater concentrations of ozone can produce reactive oxygen species (ROS), which in turn promotes the manufacture of antioxidants such AA and accounts for the first rise in its content [67]. After then, it is anticipated that the amount of AA would gradually decline as the molecule is depleted by the detoxifying and ROS scavenging processes.

Ozone application in fresh produce may induce oxidative stress and stimulate various physiological processes leading to enhanced antioxidant capacity [30], whereas both MDA and H_2O_2 are employed as stress damage indices [75]. Hydrogen peroxide content was decreased in fruits exposed to 0.2 ppm-6 h ozone compared to the fruits after 1.5 ppm-6 h ozone or control (Figure 7A). Similarly, kiwifruit fruit subjected to low ozone levels (0.3 ppm) had lower H_2O_2 content compared to untreated samples [76]. Moreover, basil bundles subjected to ozone at 1 ppm for 2 h had decreased H_2O_2 content compared to higher ozone levels [18]. Previous studies indicated H_2O_2 decline in pericarp and pulp of ozone-treated cantaloupe, whereas superoxide dismutase enzyme activity was accelerated towards oxidative stress occurrence [77].

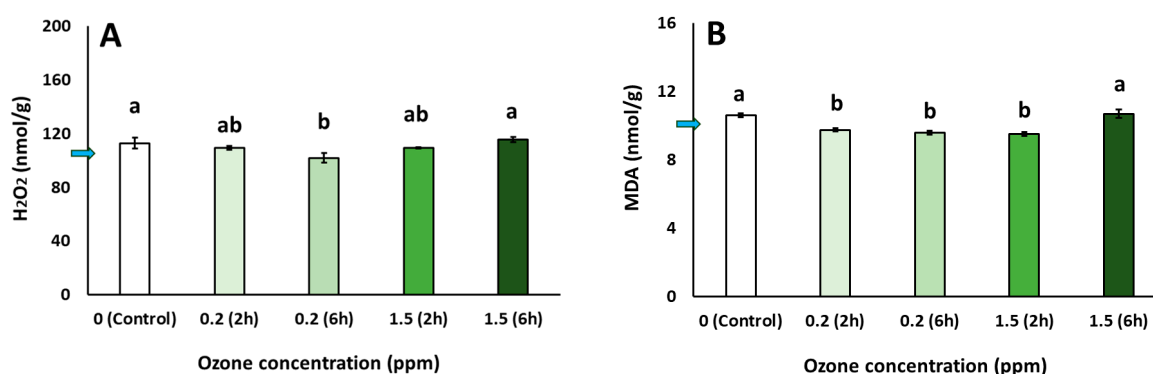


Figure 7. Impact of ozone enrichment on (A) hydrogen peroxide- H_2O_2 and (B) malondialdehyde (MDA) of cucumber fruits exposed to 0, 0.2, and 1.5 ppm ozone for 2 and 6 h and then stored for 7 days without ozone at 13 °C and RH 95%. Values represent the mean ($\pm$ standard error) of six independent replicates/treatment. The different lowercase letters indicate statistical differences ($p \leq 0.05$) among the treatments. Blue arrow indicates the values on day 0.

The MDA content was high in control and in 1.5 ppm-6 h ozone-treated fruits whereas MDA significantly decreased up to 11.6% in low ozone (i.e., 0.2 ppm) and in 1.5 ppm-2 h ozone applications compared to the control treatments (Figure 7B). Grapes subjected to

ozone at 1.5 ppm for 10 h revealed the same MDA levels as the untreated berries [78], being in agreement with the present findings from the high ozone doses and control treatment. According to earlier studies, excessive oxidative stress brought on by high ozone levels may damage the structure and permeability of cell membranes [50]. To mitigate the oxidative stress caused by ozone, kiwifruits subjected to elevated ozone levels activated a number of antioxidant enzymes, including polyphenol oxidase, superoxide dismutase, and catalase [1].

Principal component analysis (PCA) was performed to identify groups and indicate similarities and differences in multivariate data, for the 23 variables examined. The analysis of our data for ozone effects and time of exposure showed that the first eight principal components (PCs) were associated with eigenvalues higher than 1, explaining 91.9% of the cumulative variance, with PC1 accounting for 20.3%, PC2 for 18.3%, PC3 for 15.2%, PC4 for 11.9%, PC5 for 8.5%, PC6 for 7.9%, PC7 for 6.0%, and PC8 for 3.8%. The loading plot of PC1 and PC2, accounted for 38.6% of the total variation, correlated variables as follows: the upper left quadrant included appearance, ABTS, FRAP, DPPH, firmness, phenols, color a^* ; the upper right quadrant included marketability, weight loss; the lower left quadrant included respiration, H_2O_2 ; the lower right quadrant included color index, chroma, color b^* , color L^* , TSS, TSS/TA, aroma (Figure 8). However, the relevant low scores of the PC1 and PC2 together (explained 38.6% of the total variance), indicating that the variation in the dataset was distributed across multiple dimensions rather than being dominated by only two major gradients. The relatively low cumulative variance may be attributed to weak correlations among variables and the presence of several independent sources of variation.

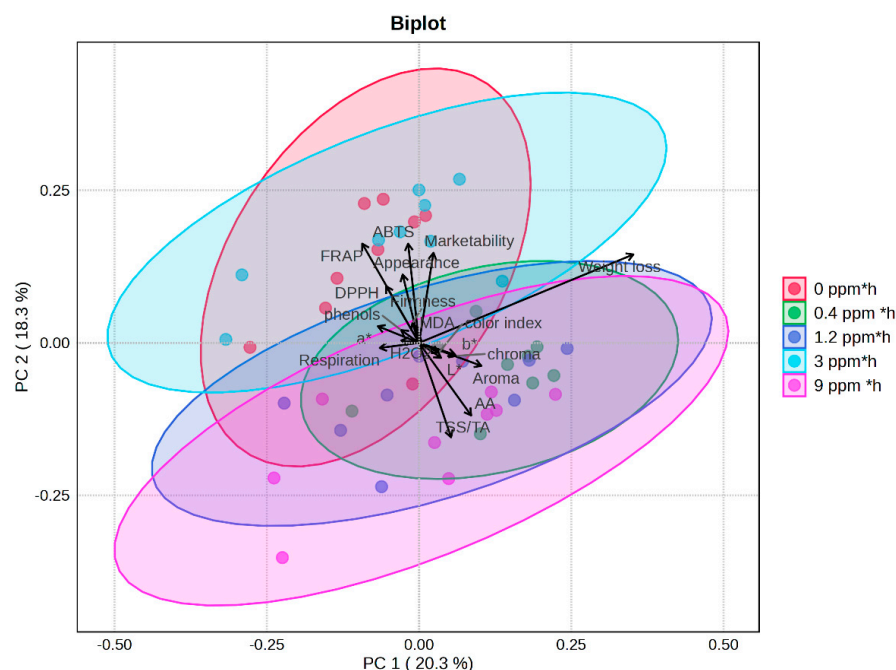


Figure 8. Loading plot of principal components 1 and 2 for ozone doses (concentration $\times$ time) factors for cucumber fruit.

PC1 reflected a ripening-related gradient. Samples with positive PC1 scores had greater TSS/TA ratios, AA content, chroma, aroma, and color index, indicating accelerated maturity and improved sensory quality. Negative PC1 scores, on the other hand, were related with increased firmness, phenolic content, and stronger antioxidant activity (DPPH, FRAP, ABTS), all of which are characteristics of less mature fruit that ripens slowly. PC2 characterized the variability associated with oxidative balance and external appearance. Positive PC2 scores were associated with increased ABTS, FRAP, appearance, and mar-

ketability, implying greater antioxidant capability and visual quality. Negative PC2 scores were associated with increased respiration, and H₂O₂, suggesting oxidative stress and pigment degradation (Figure 8).

4. Conclusions

Ozone's strong oxidative potential might result in an oxidative burst in treated fresh products, so jeopardizing the cucumbers' nutritional value. In the present study, cucumber fruit was subjected to a short-term ozone exposure at different concentrations and exposure times, examining ozone effectiveness on cucumber quality traits. The results showed that lower ozone levels (i.e., 0.2 ppm) and exposure period (2 h) were advantageous in sustaining cucumber quality-related attributes by maintaining low respiration rates. Additionally, the ozone exposure of 0.2 ppm for 2 h or 6 h resulted in greener fruits, increased ripening process and accelerated ascorbic acid, with decreased antioxidant capacity and MDA levels. In contrast, increased ozone levels of 1.5 ppm for 6 h triggered fruit respiration rates, total phenols, antioxidant capacity, and total soluble solids, in conjunction with the increased ascorbic acid content and MDA, but resulted in less marketable products. Other quality indicators, such as fruit aroma, appearance, and decay had remained unaffected by the examined treatments. Therefore, short-term ozone application may trigger fruit metabolism and boost antioxidant response to deal with the oxidative stress challenge. Application with several hours and several intermediate ozone concentrations could be studied in future research, providing additional understanding of the fresh fruit quality parameters and antioxidant content of cucumber, the metabolism limitations when triggered by an oxidative challenge such as ozone.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/horticulturae12081034/s1>, Figure S1: Impact of ozone enrichment on (A) aroma, (B) appearance, (C) marketability, and (D) decay of cucumber fruits exposed to 0, 0.2, and 1.5 ppm ozone for 2 and 6 h and then stored for 7 days without ozone at 13 °C and RH 95%.

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