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Research Article

Investigation of Different Ozone Gas Doses Treatments on Broccoli (*Brassica oleracea var. italica*) Postharvest Quality During Cold Storage

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Abstract

At postharvest, the main factor for the fruit and vegetable quality protection is to apply low storage temperature. Also, by the developing technology, alterative protection techniques start to come into prominence in addition cold storage. In a summary, the purpose of these technologies is to preserve food quality for longer time and to provide increasing of shelf-life of foods. One of the alternatives preserving technologies that has taken importance at recent years is ozone technology. In this study, different dozes of ozone gas effect on the broccoli quality were investigated. 0.05 ppm and 0.3 ppm ozone treatments were applied to samples that were kept in the vegetable compartment at refrigerator. Broccoli samples were evaluated with regards to vitamin C, chlorophyll, weight loss, total viable and mold-yeast count and visual quality parameters. As a result, it was indicated that ozone treatment has positive effects on vitamin C, chlorophyll, weight loss and visual quality during cold storage whereas hardness, total viable and mold-yeast count values do not be affected by ozone application.

Keywords: Ozone treatment, cold storage, broccoli, postharvest quality

Farklı Doz Ozon Uygulamalarının Hasat Sonrası Soğukta Saklama Sırasında Brokoli (*Brassica oleracea var. italica*) Kalitesi Üzerindeki Etkisinin İncelenmesi

Öz

Sebze ve meyveler için kalitenin sağlanmasındaki temel faktör düşük saklama sıcaklıklarının uygulanmasıdır. Bunun yanında, gelişen teknoloji ile birlikte soğukta saklamaya ek olarak alternatif koruma yöntemleri ön plana çıkmaya başlamıştır. Son yıllarda oldukça önem kazanmış alternatif koruma yöntemlerinden biri de ozon teknolojisidir. Bu çalışmada farklı doz ozon gazı konsantrasyonlarının (0,05 ve 0,3 ppm) brokolinin kalitesi üzerindeki etkisi incelenmiştir. Ev tipi buzdolaplarında sebzelik haznesine konan örneklere bir ay boyunca ozon uygulaması yapılmış ve örnekler C vitamini, klorofil, ağırlık kaybı, toplam canlı ve küf-maya sayısı ile görsel kalite parametreleri açısından değerlendirilmiştir. Buna göre ozon uygulamasının soğukta saklama sırasında ürünlerin C vitamini, klorofil, ağırlık kaybı ve görsel kalite değerleri üzerinde olumlu etkisinin olabileceği saptanırken; sertlik, renk, toplam canlı ve küf-maya sayısı değerlerinin ozonlama işleminden etkilenmediği belirlenmiştir.

Anahtar Kelimeler: Ozon uygulaması, soğukta saklama, brokoli, hasat sonrası kalite

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1. Introduction

Refrigeration is an important postharvest procedure for fruits and vegetables, which contributes to inactivation of polyphenol oxidase and inhibition of microbial growth, as well as decrease of membrane leakage, and thus can prolong shelf-life [1]. In order to preserve the freshness for a long time, low temperature storage can combine with several new technologies such as ozone gaseous treatment.

Ozonation has been used for years to disinfect water for drinking purposes. Ozone application in the food industry has not been widely used. The food industry is interested considerably in using ozone to enhance the shelf-life and safety of food products and in exploring new applications of the sanitizer. This interest was recently accompanied by an approval of ozone for the safe use, in gaseous and aqueous phases, as an antimicrobial agent on food [2,3]. Ozone is a powerful antimicrobial substance due to the its potential oxidizing capacity [4,5,6,7,8].

Ozone use may have many advantages in the food industry. Researches have shown that ozone has positive effects on food quality as well as its microbial quality [9,10,11,12,13,14,15,16,17, 18,19,20,21]. Multifunctionality of ozone application makes ozone a promising agent.

The aim of this study is to determine the effect of different dose ozone gas concentrations on the quality of broccoli during cold storage. For this purpose, 0.05 ppm and 0.3 ppm ozone treatments were applied to broccoli samples that were kept in vegetable compartment at refrigerator. The quality parameters (vitamin C, chlorophyll, weight loss, total viable count, yeast and mold count, and sensory parameters) were evaluated during storage.

2. Materials and Methods

2.1. Materials

Broccoli samples used in the study were obtained from local market. The products reached the market within one day after harvest, they were brought to laboratory on the same day. Calcium carbonate, acetone, ether, sodium sulfate, oxalic acid, 2,6 dichlorophenol-indophenol, mannitol and physiological salt solution used in the analyzes were obtained from Merck (Germany).

2.2. Ozone Application

The temperature and humidity values in the vegetable compartment of the refrigerator (Arçelik, Turkey) used in the study were continuously monitored with a data logger (Ebro logger EBI TH, Germany). The temperature and humidity profiles of the refrigerators are as given in Figure 1.

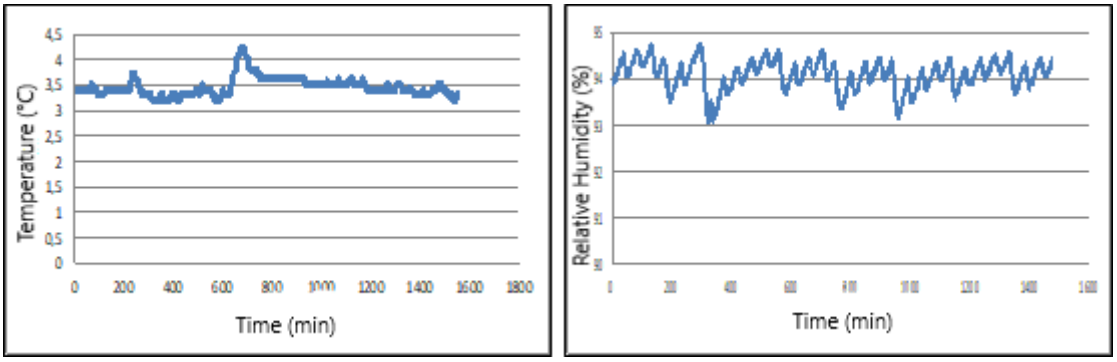

Figure 1. Temperature and humidity profiles of refrigerators used in the study

The OSHA (Occupational Safety and Health Administration) sets the limits for extended exposure and short-term exposure at 0.05 ppm and 0.3 ppm, respectively. One of the three refrigerators used in the study was chosen as the control group (no ozone applications), in others two refrigerators 0.05 ppm and 0.3 ppm ozone was applied, respectively.Ozone application was carried out with low capacity ozone (7 mg/h) generators (Onnic Inc., China). Generators are placed on the ceiling of the vegetable compartment in order to provide a more homogeneous ozone distribution in compartment.

During the storage of the products in vegetable compartment, ozone concentration was continuously monitored with an ozone analyzer (2B Technologies Brand 106-L Model, USA). Measurements were taken from the center of vegetable compartment once every ten seconds using the analyzer. In order to keep the ozone gas value at 0.05 ppm and 0.3 ppm values, the generators were operated as on/off control for certain periods with a timer during storage period. Ozone gas profiles in the vegetable compartment are given in Figure 2.

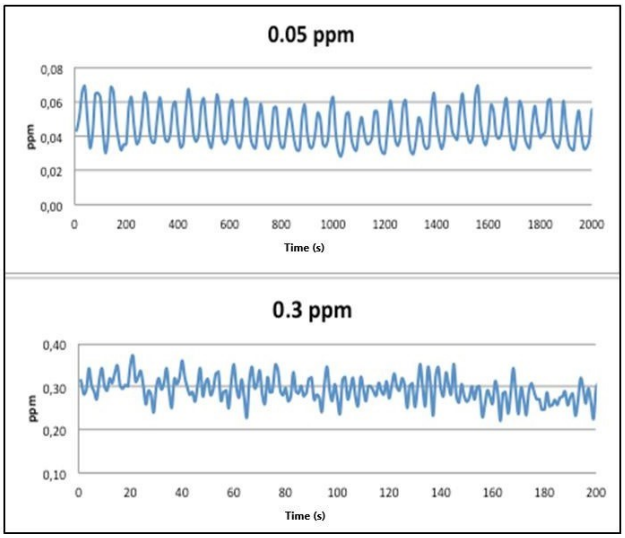

Figure 2. Ozone gas profiles in refrigerators

The samples were placed in the vegetable compartment of refrigerator by coding and ozone application started. Ozonation was continued until the end of the shelf life of the products. Physical, chemical and microbiological tests were analyzed on certain days of storage.

2.3. Vitamin C analysis

Vitamin C analysis for broccoli samples was carried out according to the spectrophotometric method. This method is based on measuring the absorbance of the excess 2,6-dichloroindophenol dye, which turns pink-purple in acid medium as a result of its reaction with ascorbic acid. It was carried out at room temperature (25°C+/-1) and under without light. Five grams of the samples to be analyzed were weighed and the weighed samples were homogenized with 20 ml of oxalic acid solution in a hand blender. The volume of the solution was made up to 50 ml with oxalic acid, shaken for 5 minutes in a shaker and centrifuged at 10000 rpm for 5 minutes. 1 ml of the supernatant from the centrifuged solution was mixed with 9 ml of the dye solution obtained by dissolving 12 mg of 2,6-dichloroindophenol sodium dihydrate in 1 liter of distilled water. The absorbance of the solution was determined by reading against the sample solution containing 1 ml of sample and 9 ml of distilled water [22].

2.4. Chlorophyll analysis

Chlorophyll measurement was carried out according to the spectrophotometric method. After 1 g of sample and 400 mg of $CaCO_3$ were homogenized with 20 ml of 85% acetone solution, it was filtered with black band filter paper and the filtrate volume was completed to 50 ml with 85% acetone solution. 20 ml of the solution was taken into a separatory funnel, 25 ml of distilled water and 30 ml of ether were added, and the aqueous phase was removed. 50 ml of distilled water and 10 ml of ether were added to the ethereal phase and agitation was carried out again in the separating funnel. The same procedure was repeated twice to remove the acetone from the solution and the aqueous phase was removed. The volume of the ethereal phase containing chlorophyll was made up to 50 ml with ether and 20 ml of the solution was taken and dried with anhydrous Na_2SO_4. Absorbance measurement was performed at 660 and 642.5 nm wavelengths. The amount of chlorophyll in mg/L was calculated according to the following equations (AOAC, 1990).

Total chlorophyll = $(7.12 \times A_{660}) + (16.8 \times A_{642.5})$ (Equation 1)
Chlorophyll a= $(9.93 \times A_{660}) - (0.777 \times A_{642.5})$ (Equation 2)
Chlorophyll b= $(17.6 \times A_{642,5}) - (2.81 \times A_{660})$ (Equation 3)

2.5. Weight loss

For weight loss evaluation, four samples were coded and placed in each of the vegetable compartment where 0.05 and 0.3 ppm ozone were applied and also control samples. In each sampling, the coded samples were weighed and placed in the vegetable compartment again. The initial weights of the samples were recorded and the weights were re-weighed at each sampling, and the weight loss was determined from the difference.

2.6. Microbiological analysis

Aseptically weighed samples (25 g) were homogenized in 225 ml physiological saline solution for one minute in a digester (IUL, S.A., Barcelona, Spain), further dilutions were made and inoculation was performed in the appropriate medium. Total mesophilic anaerobe microorganism (TMA) and mold-yeast counts were performed for both samples. The cultivated petri dishes were incubated for 48 hours at 37˚C in TMA counting and 72 hours at 28-30˚C in mold-yeast counting.

2.7. Visual evaluation

Visual evaluation tests of broccoli samples were performed by a group of 6 trained panelists. Visual quality analyzes were performed in the sensory laboratory, which conforms to the ISO 8589 standard (ISO, 1988). Broccoli removed from the vegetable compartment were immediately analyzed. Evaluation was carried out for 3 samples in each condition. Sample qualities were evaluated on a scale of 5 (5: visual quality is very good; 4: visual quality is good; 3: consumable under limited conditions; 2; visual quality is poor; 1: not consumable).

2.8. Statistical analysis

Data were collected from three independent experiments and reported as mean ± standard deviation (SD). For multiple comparisons, data were subjected to statistical analysis using Minitab (Minitab 16 Statistical Software for Windows®, Minitab Inc., Pennsylvania, USA) for the analysis of variance (ANOVA). Tukey's new multiple range test was used to analyze differences between treatments ($P < 0.05$).

3. Result and Discussion

3.1. Vitamin C content

Vitamin C is a nutrient that has a very important place in nutrition. Its concentration in foods can be affected by storage conditions such as temperature, atmospheric composition etc. Broccoli is one of the richest vegetables in terms of vitamin C content, and its vitamin content decreases during storage.

The change in vitamin C content of broccoli samples during cold storage is given in Figure 3. It is observed that the vitamin C content of broccoli samples tends to decrease during storage. At the end of the 30 days storage, the vitamin C content values of samples with 0.05 ppm and 0.3 ppm ozone gas applied and control group sample decreased by 12.3%, 8.2% and 16.7%, respectively, compared to the initial value (Table 1). Accordingly, it was observed that the highest change was observed in the control group samples during the storage period, while the least change occurred in the application of 0.3 ppm ozone gas.

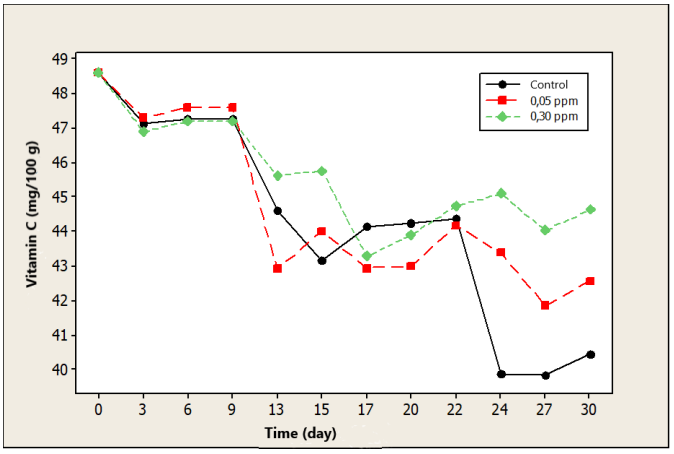

Figure 3. Changes in the content of vitamin C in broccoli samples during cold storage

According to the Tukey test, the change in vitamin C during storage was significant for all three groups ($P<0.05$). Also, a difference was found between the groups in terms of vitamin C value ($P<0.05$) (Table 1). At the end of 30 days storage, the highest vitamin C value was obtained in broccoli samples stored under 0.3 ppm ozone gas concentration.

Table 1. Vitamin C content of broccoli samples during cold storage

Day	Vitamin C (mg/100 g)*		
	0.05 ppm	**0.3 ppm**	**Control**
0	$48.6\pm0.3^{a}{}_{A}$	$48.6\pm0.3^{a}{}_{A}$	$48.6\pm0.3^{a}{}_{A}$
3	$47.3\pm0.1^{a}{}_{A}$	$46.9\pm0.7^{bc}{}_{A}$	$47.1\pm0.4^{a}{}_{A}$
6	$47.6\pm0.1^{a}{}_{A}$	$47.2\pm0.3^{ab}{}_{B}$	$47.3\pm0.0^{a}{}_{AB}$
9	$47.6\pm0.1^{a}{}_{A}$	$47.2\pm0.3^{ab}{}_{B}$	$47.3\pm0.0^{a}{}_{AB}$
13	$42.9\pm1.6^{b}{}_{B}$	$45.6\pm0.1^{cd}{}_{A}$	$44.7\pm0.1^{b}{}_{AB}$
15	$44.0\pm0.3^{b}{}_{B}$	$45.8\pm0.0^{bcd}{}_{A}$	$43.2\pm0.6^{b}{}_{C}$
17	$42.9\pm2.4^{b}{}_{A}$	$43.3\pm0.1^{f}{}_{A}$	$44.2\pm0.3^{b}{}_{A}$
20	$43.0\pm2.0^{b}{}_{A}$	$43.9\pm0.4^{ef}{}_{A}$	$44.2\pm1.2^{b}{}_{A}$
22	$44.2\pm0.8^{b}{}_{A}$	$44.8\pm0.2^{def}{}_{A}$	$44.4\pm0.7^{b}{}_{A}$
24	$43.4\pm0.2^{b}{}_{A}$	$45.1\pm0.2^{de}{}_{A}$	$39.9\pm2.4^{c}{}_{B}$
27	$41.8\pm0.0^{b}{}_{B}$	$44.0\pm1.6^{ef}{}_{A}$	$39.8\pm0.2^{c}{}_{C}$
30	$42.6\pm0.4^{b}{}_{B}$	$44.6\pm0.8^{def}{}_{A}$	$40.5\pm0.6^{c}{}_{C}$

*Values are the mean of three independent determinations ± standard deviation.
Different small letters in the same treatment during storage indicate significant differences ($P<0.05$).
Different capital letters in the same day indicate significant differences ($P<0.05$).

Previous studies have demonstrated that at harvest, vitamin C amount of broccoli was 53.34 mg/100 g. At 28 days, vitamin C amount of broccoli were 50.18 mg/100g, and 11.95 mg/100g for modified atmosphere packaged and control samples, respectively [23]. In another study examining the effect of 1-methylcyclopropene application on broccoli quality, it was revealed that the loss of vitamin C in broccoli were 30% in the samples treated with 1-

methylcyclopropene and 50% in the control group samples [24]. In a study examining the effect of ozone application on post-harvest strawberry quality, the change in ascorbic acid was found to be significant in strawberry fruit stored at 2°C for 3 days under 0.35 ppm ozone gas. At the end of cold storage, the vitamin C content was 3 times higher in the ozone-treated strawberry samples compared to the control group. Ozone can act as a phytotoxic agent due to its high oxidative capacity. In this sense, ozone and ozone-derived oxyradicals may be scavenged by low molecular weight antioxidants of the plant cell such as ascorbic acid. Changes in vitamin C contents in ozone-treated fruits could be the result of an antioxidative system that promotes the biosynthesis of vitamin C from carbohydrate reserves of the fruit [25].

3.2. Chlorophyll Content

The change in chlorophyll content of broccoli samples during cold storage is given in Figure 4. It is observed that the chlorophyll value of broccoli samples decreased during the storage period. Initial chlorophyll content of broccoli was 70.8 mg/100 g. At 30 days, chlorophyll content of broccoli was 35.5, 32.8, and 26.3 mg/100g for 0.05 ppm and 0.3 ppm ozone gas applied and control group samples, respectively (Table 2). The change in chlorophyll content according to days was statistically significant for all three groups ($P<0.05$).

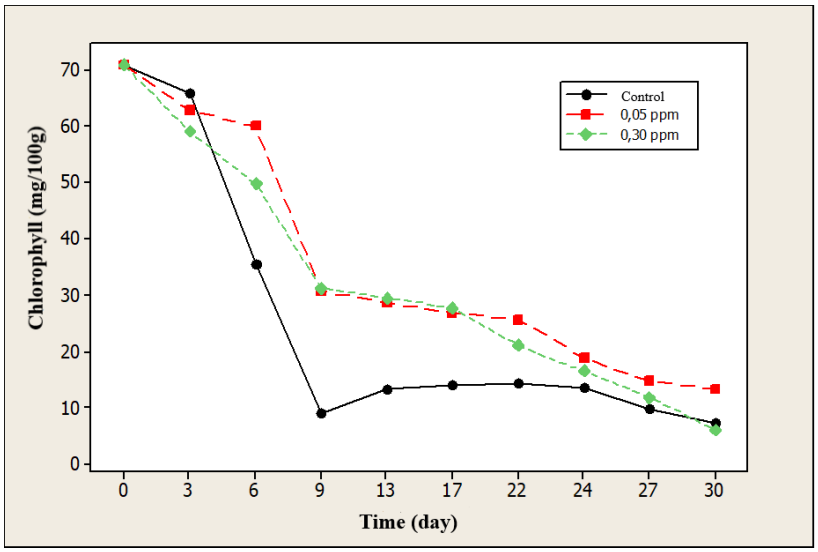

Figure 4. Changes in the content of chlorophyll in broccoli samples during cold storage

When the chlorophyll degradation between the groups is evaluated, it is seen that the most change in the chlorophyll level during the storage period occurred in the control group samples. At the end of the 9th day, a significant decrease was observed in the chlorophyll value of the control group samples, while the chlorophyll degradation was slower in the ozone-treated samples. Chlorophyll content of ozone-treated broccoli samples was found to be statistically different from untreated control group samples ($P<0.05$).

Table 2. Chlorophyll content of broccoli samples during cold storage

Day	Chlorophyll (mg/100 g)*		
	0.05 ppm	**0.3 ppm**	**Control**
0	$70.8\pm3.3^{a}{}_{A}$	$70.8\pm3.3^{a}{}_{A}$	$70.8\pm3.3^{a}{}_{A}$
3	$62.8\pm3.4^{b}{}_{AB}$	$59.0\pm1.3^{b}{}_{B}$	$66.0\pm0.6^{a}{}_{A}$
6	$60.0\pm2.5^{b}{}_{A}$	$49.8\pm0.1^{c}{}_{B}$	$35.4\pm1.5^{b}{}_{C}$
9	$30.8\pm2.7^{c}{}_{A}$	$31.2\pm3.7^{d}{}_{A}$	$9.0\pm2.5^{cd}{}_{B}$
13	$28.6\pm2.3^{c}{}_{A}$	$29.5\pm2.7^{de}{}_{A}$	$13.2\pm3.0^{cd}{}_{B}$
17	$26.9\pm4.9^{ca}{}_{A}$	$27.7\pm5.4^{de}{}_{A}$	$14.0\pm2.0^{c}{}_{B}$
22	$25.0\pm3.5^{cd}{}_{A}$	$21.1\pm5.4^{ef}{}_{AB}$	$14.2\pm3.2^{c}{}_{B}$
24	$18.8\pm3.5^{de}{}_{A}$	$16.5\pm3.8^{fg}{}_{A}$	$13.6\pm1.7^{c}{}_{A}$
27	$14.8\pm1.5^{e}{}_{A}$	$11.9\pm0.8^{gh}{}_{B}$	$9.8\pm1.1^{cd}{}_{C}$
30	$13.2\pm0.4^{e}{}_{A}$	$6.1\pm0.4^{h}{}_{B}$	$7.4\pm1.9^{d}{}_{B}$

*Values are the mean of three independent determinations ± standard deviation.
Different small letters in the same treatment during storage indicate significant differences ($P<0.05$).
Different capital letters in the same day indicate significant differences ($P<0.05$).

Previous studies have shown that the chlorophyll content of broccoli decreases over time due to enzyme activity, both during at room temperature or cold storage [26,27]. Forney et al. [28], in their study examining the effect of 0.2 ppm and 0.7 ppm ozone gas concentrations on the chlorophyll content of broccoli, stated that the yellowing of broccoli may be caused by ethylene. The reduction of yellowing of broccoli samples may be related to inhibition of chlorophyll degrading enzymes and/or the induction of antioxidants that can protect chlorophyll. Same research suggest that lipid peroxidation may be responsible for chlorophyll loss during broccoli senescence. As a response to ozone, broccoli may increase levels of antioxidant chemicals and enzymes which could slow chlorophyll loss. Karaca and Velioğlu [29] stated that 950 µL/L ozone concentration had no effect on chlorophyll content of parsley. Sevilgen [30] reported that the decrease in the amount of chlorophyll a and chlorophyll b in chard increased with the increase of ozone dose. It concluded that 20 g/hour ozone application is more effective on the decrease in the amount of chlorophyll a and chlorophyll b compared to disinfectant applications such as chlorine and hydrogen peroxide.

3.3. Weight Loss

The changes in weight loss of broccoli sample during cold storage are shown in Figure 5. It is observed that the weight value of the products decreases due to moisture loss during the storage period. During the storage period, the changes in the weight values of 0.05 ppm, 0.3 ppm and control group samples were obtained as 9.4%, 9.3% and 16.6%, respectively (Table 3).

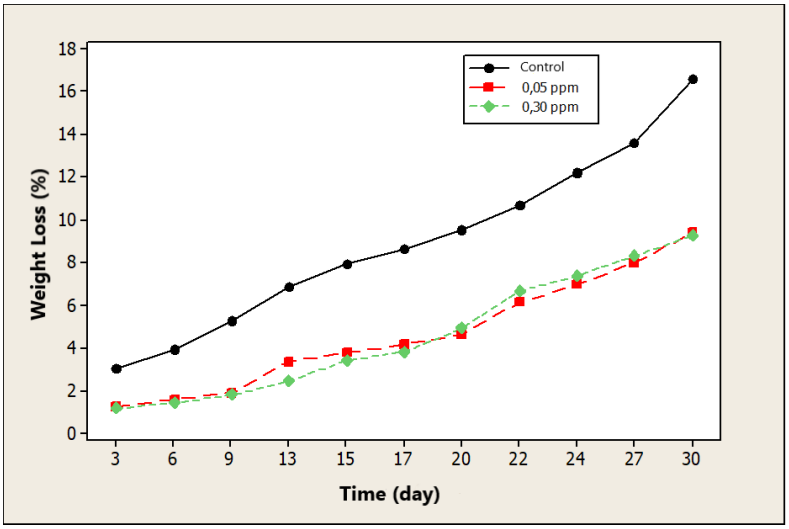

Figure 5. Changes in weight loss in broccoli samples during cold storage

Weight loss values according to days were statistically significant for each group (P<0.05). It also differs statistically between the ozone-treated and untreated samples. It was observed that the weight loss value was similar under the conditions of 0.05 ppm and 0.3 ppm ozone application, but higher in the control group samples than in the ozone groups.

Table 3. Weight loss values of broccoli samples during cold storage

Day	Weight Loss (%)*		
	0.05 ppm	0.3 ppm	Control
3	$1.3\pm0.0^{f}{}_{B}$	$1.2\pm0.1^{e}{}_{B}$	$3.1\pm0.3^{i}{}_{A}$
6	$1.6\pm0.0^{f}{}_{B}$	$1.4\pm0.2^{de}{}_{B}$	$3.9\pm0.1^{hi}{}_{A}$
9	$1.9\pm0.2^{ef}{}_{B}$	$1.8\pm0.1^{de}{}_{B}$	$5.3\pm0.4^{gh}{}_{A}$
13	$8.3\pm0.8^{def}{}_{B}$	$9.6\pm0.2^{cde}{}_{B}$	$9.8\pm09^{fg}{}_{A}$
15	$3.8\pm0.9^{de}{}_{B}$	$3.4\pm0.6^{bcde}{}_{B}$	$8,0\pm0,8^{ef}{}_{A}$
17	$4.2\pm0.8^{cd}{}_{B}$	$3.8\pm0.7^{bcde}{}_{B}$	$8.7\pm0.6^{def}{}_{A}$
20	$4.7\pm0.8^{cd}{}_{B}$	$5.0\pm0.9^{abcde}{}_{B}$	$9.6\pm0.1^{de}{}_{A}$
22	$6.2\pm0.4^{bc}{}_{B}$	$6.7\pm1.6^{abcd}{}_{B}$	$10.7\pm0.3^{cd}{}_{A}$
24	$7.0\pm0,5^{b}{}_{B}$	$7.4\pm1.9^{abc}{}_{AB}$	$12.2\pm0.5^{bc}{}_{A}$
27	$8.0\pm0.3^{ab}{}_{A}$	$8.3\pm1.3^{ab}{}_{A}$	$13.6\pm0.8^{b}{}_{A}$
30	$9.4\pm0.1^{a}{}_{B}$	$9.3\pm1.5^{a}{}_{B}$	$16.6\pm0.2^{a}{}_{A}$

*Values are the mean of three independent determinations ± standard deviation.
Different small letters in the same treatment during storage indicate significant differences (P<0.05).
Different capital letters in the same day indicate significant differences (P<0.05).

Forney et. al. [28] stated that 0.7 ppm ozone gas application increased weight loss in broccoli. The samples applied with 0.7 ppm ozone gas had 24% weight loss, while the samples applied with 0.2 ppm ozone gas and the control group had similar weight loss values with each other. The increase in weight loss in high dose (0.7 ppm) application was associated with the damage caused by ozone in the sample tissues. Ozone induced membrane damage, which causes increased membrane leakiness, could also contribute to enhanced water loss. Nadas et al. [31] reported that strawberries applied with 1.5 ppm ozone had a lower weight loss during storage

at 2ºC compared to the control group. It can be explained by the fact that ozone affects perspiration and reduces water loss. Geransayeh et. al. [32] found that the weight loss in grape samples treated with water containing 0.3 ppm ozone was 0.06% less than in the control group samples. In the same research, it was also stated that ozone used in high concentration may increase weight loss due to damage to the tissue.

3.4. Microbiological Analysis

The results of Tukey Multiple Range tests based on the analysis of variance are presented in Table 4. Ozone treatment at the level of 0.05 and 0.3 ppm and control group increase the total mesophilic microorganism counts of broccoli with initial values of 2.8 to 5.8; 2.6 to 5.3 and 2.6 to 5.3 log cfu/ml, respectively. Statistical analyses showed that the concentration levels can be considered significantly not different at the 0.05 confidence level. This is consistent with the observations of Chen et al. [17]. They found that ozone treatment failed to reduce the microbial populations at low concentrations during storage of cantaloupes. Allende et. al. [33] observed that strawberries stored for 12 days in an atmosphere containing 5000 mg ozone/L did not differ in the final counts of mesophilic and psychrophilic bacteria, molds and yeasts compared to the untreated fruits.

Table 4. Effect of ozone concentration on microorganism count on broccoli (log cfu/g)

Day	Total Viable Microorganism Count (log cfu/ml)*		
	0.05 ppm	**0.3 ppm**	**Control**
0	$2.8\pm0.4^{c\ A}$	$2.6\pm2.1^{c\ A}$	$2.6\pm0.1^{b\ A}$
6	$3.6\pm0.4^{bc\ A}$	$3.6\pm0.8^{bc\ A}$	$2.8\pm0.3^{b\ A}$
13	$4.6\pm0.4^{abc\ A}$	$5.7\pm0.5^{a\ A}$	$5.0\pm0.2^{a\ A}$
16	$4.7\pm0.4^{abc\ A}$	$5.8\pm0.1^{a\ A}$	$5.6\pm1.0^{a\ A}$
20	$5.8\pm0.6^{a\ A}$	$6\pm0.1^{a\ A}$	$5.7\pm0.3^{a\ A}$
22	$5.3\pm0.4^{ab\ A}$	$5.4\pm0,6^{a\ A}$	$5.8\pm0.6^{a\ A}$
26	$4.6\pm0.9^{abc\ A}$	$4,8\pm0.2^{ab\ A}$	$4.5\pm0.6^{ab\ A}$
30	$5.8\pm0.3^{a\ A}$	$5.3\pm0.4^{a\ A}$	$5.3\pm0.3^{a\ A}$

*Values are the mean of three independent determinations ± standard deviation.
Different small letters in the same treatment during storage indicate significant differences (P<0.05).
Different capital letters in the same day indicate significant differences (P<0.05).

Ozone is a compound with antimicrobial effect. Molecular ozone affects the membrane permeability of microorganism cells, which causes the reaction of intracellular components and ultimately cell death [34]. In a study on the reduction of microbial load in date fruit by ozone application, the effectiveness of ozone gas was tested at 1, 3 and 5 ppm concentrations and at 15, 30, 45, and 60 minutes application times. Accordingly, it was observed that the total number of mesophilic microorganisms decreased after 1 hour of application of 1, 3 and 5 ppm concentrations from the initial value of 4.06 cfu/g to 3.8, 3.6 and 3.5 log cfu/g, respectively. This difference between the groups was found to be statistically significant. In the same study, it was stated that a higher rate of microbial reduction could be achieved by increasing the application time (3 or 5 hours) [35]. In another research, the total mesophilic bacteria count of

dried fig samples with 13.8 mg/L ozone application after 7.5, 15 and 30 minutes was found to reduce to 0.81, 1.0 and 1.42 log cfu/g, respectively [36]. It was seen that the 0.05 ppm and 0.3 ppm ozone gas concentrations tested in this experimental study were not sufficient to reduce the total number of microorganisms in broccoli. In order to achieve an effective microbial reduction, higher concentrations should be tried.

The change in the mold-yeast count of broccoli samples during the 30-day storage period is shown in Figure 6. No significant change was observed in the number of mold-yeast according to the days.

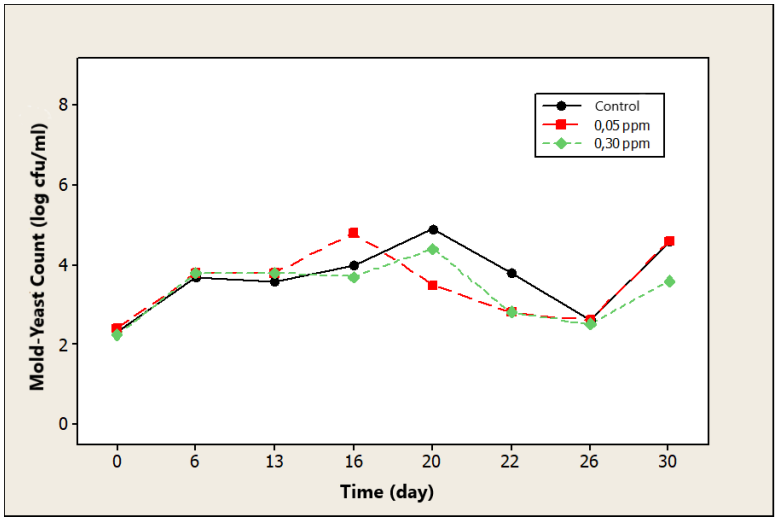

Figure 6. Change in mold-yeast count in broccoli samples during storage

The initial mold-yeast count, which was 2.4, 2.3 and 2.3 log cfu/mL for the samples treated with 0.05 ppm and 0.3 ppm ozone and the control group, was changed to 4.6, 3.6 and 4.6 log cfu/mL at the end of the 30-day cold storage, respectively (Table 5). There was no statistically significant difference between the groups for the mold-yeast count value (P>0.05). Wang et. al [37] treated grape tomatoes with 1.71, 3.43 and 6.85 mg/L gaseous ozone for 2 or 4 hours. They found that ozone application no impact on yeasts and molds.

Table 5. Mold-yeast count in broccoli samples during storage

Day	Mold-Yeast Count (log cfu/ml)*		
	0.05 ppm	**0.3 ppm**	**Control**
0	$2.4\pm0.1^{d\ A}$	$2.3\pm0.2^{b\ A}$	$2.3\pm0.1^{b\ A}$
6	$3.8\pm0.1^{ab\ A}$	$3.8\pm0.0^{ab\ A}$	$3.7\pm0.4^{ab\ A}$
13	$3.8\pm0.1^{ab\ A}$	$3.8\pm0.6^{ab\ A}$	$3.6\pm0.6^{ab\ A}$
16	$4.8\pm0.3^{a\ A}$	$3.7\pm0.3^{ab\ A}$	$4.0\pm0.7^{ab\ A}$
20	$3.5\pm0.3^{bc\ A}$	$4.4\pm1.0^{a\ A}$	$4.9\pm0.3^{a\ A}$
22	$2.8\pm0.3^{bcd\ A}$	$2.8\pm0.3^{ab\ A}$	$3.8\pm0.3^{ab\ A}$
26	$2.6\pm0.3^{cd\ A}$	$2.5\pm0.3^{b\ A}$	$2.6\pm0.6^{b\ A}$
30	$4.6\pm0.4^{a\ A}$	$3.6\pm0.3^{ab\ A}$	$4.6\pm0.6^{a\ A}$

*Values are the mean of three independent determinations ± standard deviation.
Different small letters in the same treatment during storage indicate significant differences ($P<0.05$).
Different capital letters in the same day indicate significant differences ($P<0.05$)

Similar to the total viable microorganism, ozone is known to be an effective disinfectant in reducing the number of molds and yeasts. In the studies, it was found that the initial mold-yeast number of date fruit, which was found as 3.9 log cfu/g, decreased with ozone application. As a result of 1-hour ozone application at 1, 3 and 5 ppm concentrations, mold-yeast numbers were found to be 3.8, 3.6 and 3.5 log cfu/g, respectively. In the same study, it was stated that lower concentrations should be applied for a longer time or higher concentrations should be applied for a shorter time in order to reduce mold/yeast activity [34]. It is stated in the literature that ozone gas is effective in fungal deterioration for different application conditions. Accordingly, 15% less fungal spoilage was observed in strawberries treated with 0.35 ppm ozone gas compared to untreated products [25]. It is seen that a higher rate of fungal reduction will be achieved by increasing the ozone concentration. It is seen that the 0.05 ppm and 0.3 ppm ozone gas concentrations applied in this study are not at an enough level to reduce the mold-yeast level in broccoli.

3.5. Visual Assessment

Visual quality of broccoli samples in all three groups decreased during storage (Figure 7). The visual quality value, which was 5 for the control group and the samples stored under 0.05 ppm and 0.3 ppm ozone gas on day 0, drops below the limit value of 3 after the 17th day in the samples stored under 0.3 ppm ozone gas. While the control group samples drop below the limit value of 3 at the end of the 23rd day, it was observed that the samples stored under 0.05 ppm ozone gas were still at the limit value at the end of the 30th day (Figure 7).

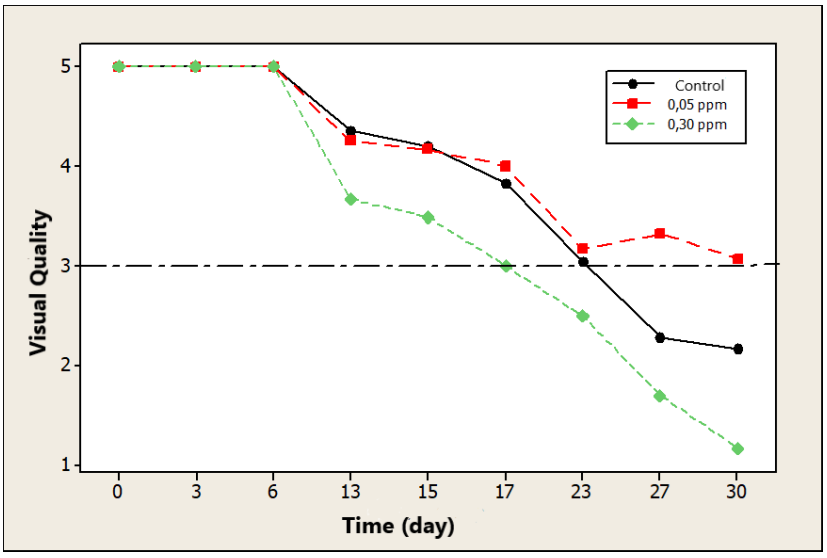

Figure 7. Visual quality of broccoli during storage

In previous studies about the quality of broccoli, it was observed that the samples stored in refrigerator conditions with a temperature of 1-2ºC became unconsumable at the end of the 20th day, similar to the results obtained for the control group in this research [38]. The main reasons for the difference between the groups are browning/color changes on the broccoli samples under 0.3 ppm ozone concentration and textural changes such as softening and shrinking occur in the control group samples due to weight loss.

During sensory evaluation tests, it was determined that high ozone concentration caused browning on the sample. The Tukey comparison test results of the samples evaluated in terms of the browning parameter (5: No browning; 3: Moderate browning; 1: Severe browning) are given in Table 6. It was observed that visually severe browning was occurred in 0.3 ppm ozone samples during storage, this difference becomes statistically significant from the 27th day of storage (P<0.05).

Tablo 6. Visual browning value of broccoli during storage

Day	Visual Browning Value*		
	0.05 ppm	**0.3 ppm**	**Control**
0	$5.0\pm0.0^{a}{}_{A}$	$5.0\pm0.0^{a}{}_{A}$	$5.0\pm0.0^{a}{}_{A}$
3	$4.7\pm0.3^{a}{}_{AB}$	$4.2\pm0.3^{ab}{}_{B}$	$5.0\pm0.0^{a}{}_{A}$
6	$4.7\pm0.3^{a}{}_{A}$	$3.9\pm0.4^{abc}{}_{A}$	$4.7\pm0.3^{ab}{}_{A}$
13	$4.7\pm0.3^{a}{}_{A}$	$3.9\pm0.4^{abc}{}_{A}$	$4.7\pm0.3^{ab}{}_{A}$
15	$4.4\pm0.6^{ab}{}_{A}$	$3.7\pm0.8^{abcd}{}_{A}$	$4.6\pm0.3^{ab}{}_{A}$
17	$4.0\pm0.4^{abc}{}_{A}$	$3.1\pm1.0^{bcd}{}_{A}$	$4.0\pm0.2^{b}{}_{A}$
23	$3.4\pm0.4^{bc}{}_{A}$	$2.3\pm0.9^{cd}{}_{A}$	$3.2\pm0.3^{c}{}_{A}$
27	$3.4\pm0.3^{bc}{}_{A}$	$2.1\pm0.5^{d}{}_{B}$	$3.1\pm0.1^{c}{}_{A}$
30	$3.1\pm0.3^{c}{}_{A}$	$2.0\pm0.5^{d}{}_{A}$	$3.1\pm0.3^{c}{}_{B}$

*Values are the mean of three independent determinations ± standard deviation.
Different small letters in the same treatment during storage indicate significant differences (P<0.05).
Different capital letters in the same day indicate significant differences (P<0.05).

The results obtained in this research on visual quality are similar to the literature. It is mentioned in the literature that ozone gas can cause color change/darkening in products. The decomposition of ozone gas in the cell wall and plasma membrane increases the membrane permeability. This causes the loss of cellular components and an increase in the activity of oxidative enzymes [39].

4. Conclusion

In this study, the effects of different doses of ozone gas concentrations on the quality of broccoli during postharvest cold storage were revealed.

It was observed that the loss of vitamin C in broccoli samples stored under 0.05 and 0.3 ppm ozone gas, which was continuously applied to the vegetable compartment of the refrigerator, was lower than the control group that was not treated. In addition, due to the inactivation of enzymes that cause chlorophyll degradation by ozone, chlorophyll loss in the ozone-treated samples during the storage period was lower than in the control group. In this research, it was concluded that ozone has a reducing effect on weight loss of broccoli samples. The effect of ozone gas on the natural microflora of broccoli was evaluated in this study. It was found that the total viable microorganism and mold-yeast levels of broccoli samples with applying 0.05 ppm and 0.3 ppm ozone gas and samples without applying ozone gas were similar. As a result of the sensory evaluations, it was observed that the visual quality was decreased during the 30-

day storage period. Visual quality was decreased below the limit value of 3 after the 17th day in the samples stored under 0.3 ppm ozone gas, and after the 23rd day in the control group samples. It was found that the samples stored under 0.05 ppm ozone gas were still at the limit value at the end of the 30th day in terms of visual quality.

Overall, 0.05 ppm ozone gas concentration which is continuously applied in the vegetable compartment of refrigerator was found to be optimum treatment to maintain, improve postharvest quality and prolonging the shelf life of broccoli.

Ethics in Publishing

There are no ethical issues regarding the publication of this study.

Author Contributions

Gönül Çavuşoğlu Kaplan: Drafted and wrote the manuscript, performed the experiment and result analysis.

Ebru Fıratlıgil: Assisted in analytical analysis on the structure, supervised the experiment's progress, result interpretation and helped in manuscript preparation.

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References

[1] Li, P., Yu, X., Xu, B. (2017) Effects of UV-C light exposure and refrigeration on phenolic and antioxidant profiles of subtropical fruits (Litchi, Longan, and Rambutan) in different fruit forms, Journal of Food Quality, Article ID 8785121, 12 pages.

[2] Kim, J.G., Yousef, A.E., Khadre, M.A. (2003) Ozone and its current and future application in food industry, Advances in Food and Nutrition Research, 45: 167-218.

[3] Guzel-Seydim, Z.B., Greene, A.K., Seydim, A.C. (2004) Use of ozone in the food industry, LWT-Food Science and Technology, 34(4): 453-460.

[4] Kuşçu, A. and Pazır, F. (2004) Ozone applications in food industry, Gıda, 29 (2): 123-129. (in Turkish)

[5] Tzortzakis, N. and Sargyris, A. (2017) Postharvest ozone application for the preservation of fruits and vegetables, Food Reviews International, 33(3): 270-315.

[6] Baia, G.M., Freitas-Silva, O., Junior, M.F. (2020) Understanding the role of chlorine and ozone to control postharvest diseases in fruit and vegetables: A review, Current Nutrition & Food Science, 16(4): 455-461.

[7] Botondi, R., Barone, M., Grasso, C. (2021) A review into the effectiveness of ozone technology for improving the safety and preserving the quality of fresh-cut fruits and vegetables, Foods, 10(4): 748.

[8] Sarron, E., Gadonna-Widehem, P., Aussenac, T. (2021) Ozone treatments for preserving fresh vegetables quality: A critical review, Foods, 10(3): 605.

[9] Achen, M. and Yousef, A.E. (2001) Efficacy of ozone against *Escherichia coli O157:H7* on apples, Journal of Food Science, 66(9): 1380-1384.

[10] Salvador, A., Abad, I., Arnal, L., Martinez-Javega, J.M. (2006) Effect of ozone on postharvest quality of persimmon, Journal of Food Science, 71(6): S443-S446.

[11] Akbas, M.Y. and Ozdemir, M. (2008) Effect of gaseous ozone on microbial inactivation and sensory of flaked red peppers, International Journal of Food Science and Technology, 43(9): 1657–1662.

[12] Ölmez, H. and Akbaş M. (2008) Optimization of ozone treatment of fresh-cut green leaf lettuce, Journal of Food Engineering, 90(4): 487–494.

[13] Zambre, S.S., Venkatesh, K.V., Shah, N.G. (2010) Tomato redness for assessing ozone treatment to extend the shelf life, Journal of Food Engineering, 96(3): 463–468.

[14] Karaca, H., Spencer S. Walseb, S.S., Smilanick, J.L. (2012) Effect of continuous 0.3 µL/L gaseous ozone exposure on fungicide residues on table grape berries, Postharvest Biologyand Technology, 64(1): 154–159.

[15] Kim, C. and Hung, Y. (2012) Inactivation of *E. coli O157:H7* on blueberries by electrolyzed water, ultraviolet light, and ozone, Journal of Food Science, 77(4): M206-M211.

[16] Evrendilek, G.A and Uzun, K.K. (2020) Effects of ozonation, alkaline ionized water, and their combination on surface disinfection and shelf life extension of tomatoes, Journal of Food Processing and Preservation, 44(10): e14792.

[17] Chen, C., Zhang, H., Zhang, X., Dong, C., Xue, W., Xu, W. (2020) The effect of different doses of ozone treatments on the postharvest quality and biodiversity of cantaloupes, Postharvest Biology and Technology, 163: 111124.

[18] Bigi, F., Haghighi, H., Quarteri, A., Leo, D. R., Pulvirenti, A. (2021) Impact of low-dose gaseous ozone treatment to reduce the growth of in vitro broth cultures of foodborne pathogenic/spoilage bacteria in a food storage chamber, Journal of Food Safety, 41(3): e12892.

[19] Panau, A.A., Akrida-Demertzi, K., Demertzis, P., Riganakos, K.A. (2021) Effect of gaseous ozone and heat treatment on quality and shelf life of fresh strawberries during cold storage, International Journal of Fruit Science, 21(1): 218-231.

[20] Gorzelany, J., Kapusta, I., Zardzewialy, M., Belcar, J. (2022) Effects od ozone application on microbiological stability and content of sugars and bioactive compounds in the fruit of Saskatoon berry (*Amelanchier alnifolia Nutt.*), Molecules, 27(19): 6446.

[21] Kaur, P., Kaur, K., Devgan, K., Kumar, M., Sandhu, K., Kaur, A. (2022) Potential of low-dose aqueous ozone treatment and packaging to extent quality and shelf-life of green pea pods under cold storage, Journal of Food Processing and Preservation, 46(10): e16165.

[22] Hışıl, Y., (1993) Enstrümental Gıda Analizleri Laboratuar Kılavuzu, EgeÜniversitesi, Mühendislik Fakültesi Çoğaltma Yayınları, No:55, İzmir, (45)s.

[23] Sabir, F.K. (2012) Postharvest quality response of broccoli florets to combined application of 1-methylcyclopropene and modify atmosphere packing, Agricultural and Food Science, 21(4): 421-429.

[24] Yuan, G., Sun, B., Yuan, J., Wang, Q. (2010) Effect of 1-methlycyclopropene on shelf life, visual quality, antioxidant enzymes and health-promoting compounds in broccoli florets, Food Chemistry, 118(3): 774-781.

[25] Perez, A.G., Sanz, C., Rios, J.J., Olias, R., Olias, J.M. (1999) Effects of ozone treatment on postharvest strawberry quality, Journal Agric. Food Chem., 47(4): 1652−1656.

[26] Aiamla-or, S., Yamauchi, N., Takino, S., Shigyo, M. (2009) Effect of UV-A and UVB irradiation on broccoli (*Brassica oleracea L. Italica Group*) floret yellowing during storage, Postharvest Biol. Technol., 54(3): 177-179.

[27] Nath, A., Bagchi, B, Misra, L.K., Deka, B.C. (2011) Changes in post-harvest phytochemical qualities of broccoli florets during ambient and refrigerated storage, Food Chem., 127(4): 1510-1514.

[28] Forney, C.F., Song, J., Fan, L.F., Hildebrand, P.D., Jordan, M.A. (2003) Ozone and 1-methylcyclopropene slter the postharvest quality of broccoli, J. Amer. Soc. Hort. Sci., 128(3): 403-408.

[29] Karaca, H. and Velioğlu, Y.S. (2014) Effects of ozone treatments on microbial quality and some chemical properties of lettuce, spinach, and parsley, Postharvest Biology and Technology, 88:46–53.

[30] Sevilgen, Ö. (2009) Ozon, klor ve hidrojen peroksit uygulamalarının pazıda klorofil miktarı üzerine etkileri, Yüksek Lisans Tezi, Ankara Üniversitesi.

[31] Nadas, A., Olmo, M., and Garcia, J. M. (2003) Growth of *Botrytis cinerea* and strawberry quality in ozone-enriched atmospheres, J. Food Sci. 68(5):1798-1802.

[32] Geransayeh, M., Mostofi, Y., Abdossi, V., Nejatian, M.A. (2012) Effects of ozonated water on storage life and postharvest quality of Iranian table grape (*cv Bidaneh Qermez*), Journal of Agricultural Science, 4(2): 31-38.

[33] Allende, A., Marin, A., Buendia, B., Tomas-Barberan, F., Gil, M.I. (2007) Impact of combined postharvest treatments (UV-C light, gaseous O_3, superatmospheric O_2 and high CO_2) on health promoting compounds and shelf-life of strawberries, Postharvest Biol. Technol., 46: 201–211.

[34] Ölmez, H. (2012) Ozone in decontamination of fresh and minimally processed produce, Edited by V.M. Gómez-López. pp 177-194. John Wiley & Sons, Inc.

[35] Najafi, M.B.H and Khodaparast, M.H. H. (2009) Efficacy of ozone to reduce microbial populations in date fruits, Food Control, 20(1): 27–30.

[36] Zorlugenç, B., Zorlugenç, F.K., Öztekin, S., Evliya, İ.B. (2008) The influence of gaseous ozone and ozonated water on microbial flora and degradation of aflatoxin B_1 in dried figs, Food and Chemical Toxicology, 46(12): 3593-3597.

[37] Wang, E., Fan, X., Sokorai, K., Sites, J. (2019) Quality deterioration of grape tomato fruit during storage with gaseous ozone at conditions that significantly reduced populations of *Salmonella* on stem scar and smooth surface, Food Control, 103: 9-20.

[38] Fernández-León, M.F., Fernandez-Leon, A.M, Lozano, M., Ayuso, M.C., Gonzalez-Gomez, D. (2013) Different postharvest strategies to preserve broccoli quality during storage and shelf life: Controlled atmosphere and 1-MCP, Food Chemistry, 138 (1): 564–573.

[39] Skog, L.J. and Chu, C.L. (2001) Effect of ozone on qualities of fruits and vegetables in cold storage, Canadian Journal of Plant Science, 81(4): 773-778.