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Gaseous Ozone and Citric Acid on Quality and Shelf-Life of Lettuce Packaged Under Passive Modified Atmosphere During Cold Storage

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Abstract: Ozone is considered a promising food preservation technique and has gained great interest because of its strong oxidizing properties and significant antimicrobial efficacy, and because its decomposition leaves no residue in food. In the present study the combined effect of ozone and citric acid treatment on quality and shelf life of freshly cut lettuce packaged under a passive modified atmosphere during cold storage was investigated. Lettuce were washed, cut and treated with ozone at 0.5, 1.0 or 1.5 ppm for 45 minutes. Another batch was treated with citric acid and 3 additional batches were treated with ozone at the above concentrations plus citric acid. Headspace analysis, color parameters, pH, microbiological and sensory analysis were monitored during cold storage. Carbon dioxide production rates increased significantly ($p<0.05$) in ozonated lettuce at 0.5 and 1.0 ppm compared to the control. Ozone treatment at 0.5 and 1.0 ppm and 0.5 ppm plus citric acid caused a significant decrease ($p<0.05$) in total mesophilic counts, L* and a* color parameters compared to the control. No treatments significantly ($p <0.05$) affected yeast and mold counts. Treatment with citric acid caused a significant ($p <0.05$) decrease in pH compared to the control. Ozone treatment at 0.5 ppm and 0.5 ppm plus citric acid extended shelf-life of lettuce by 4 days compared to the untreated samples during cold storage.

Keywords: ozone; citric acid; lettuce; shelf-life; cold storage

1. Introduction

There is a demand for production of organic products of the same quality as conventionally produced crops. Freshly harvested or minimally processed vegetables are susceptible to development of the pathogenic bacteria *Escherichia coli O157:H7*, *Escherichia coli O104:H4*, *L.monocytogenes*, *Shigella*, *Salmonella*, and *Pseudomonas fluorescens* during cultivation or after harvest [1 – 7]. Food processors look for effective sanitation techniques to improve safety and quality of freshly cut vegetables [8, 9].

Ozone (O3, 48 g/mol) is a highly unstable triatomic molecule of oxygen (O), with the oxygen atoms arranged to form an obtuse angle. In nature, ozone is formed during lightning discharges and from ultraviolet radiation from the sun, so it appears in the earth's atmosphere in gaseous form and in very low concentrations. The mechanism of ozone formation involves the splitting of diatomic oxygen molecules (O2) into single oxygen atoms (O) using a significant input of energy. Single oxygen atoms quickly combine with O2 to form the highly reactive O3 molecule. Ozone acts as a powerful disinfectant against viruses, bacteria and fungi because of the three oxygen atoms [10].Ozone was identified as a "generally recognized as safe" substance [11]. This has allowed it to be used in the food industry especially for minimally processed fresh vegetables. Ozone has been used for sanitation of food plant equipment and waste water reuse [12, 13] and in sanitation of fruits and of freshly cut or minimally processed vegetables [8, 9, 13- 19]. Ozonation does not leave chemical residues [20] and does not alter sensory characteristics of products [14].

In a previous work, we studied the effect of gamma radiation on sensory characteristics, physicochemical parameters and shelf life of strawberries during cold storage [21]. Also, in a more recent study, the effect of combined gaseous ozone and heat treatment on basic physicochemical and

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sensory characteristics and shelf life of strawberries stored under refrigerated conditions was studied [22]. The purpose of the present study was to investigate effects of ozone alone or combined with citric acid on chemical, microbiological and sensory characteristics of freshly cut lettuce packaged under passive modified atmosphere, to determine if shelf-life can be extended.

2. Materials and Methods

2.1. Experimental Design

Fifteen kg of freshly harvested lettuce was obtained from the local vegetable market and immediately transferred to the Food Chemistry Laboratory of the Department of Chemistry, University of Ioannina. Cores were excised and discarded and the lettuce leaves were chopped with a sterile, sharp and stainless knife then washed with tap water and drained using a centrifugal kitchen dryer. The leaves were thoroughly blended to achieve a uniform mix and divided into 8 equal batches. Tests were performed to select appropriate conditions for experiments. Two bags of 2 different laminated PA//LLDPE and PET//LLDPE plastic films were used to determine which was most suitable for maintaining quality characteristics of fresh lettuce packaged under passive modified atmosphere during cold storage. The best results were with the PET//LLDPE film. Five immersion times of lettuce leaves (5 minutes, 3 minutes, 1 minute, 45 seconds or 30 seconds) in a 1% w / v citric acid solution were tested to achieve maximum retention of sensory characteristics and the greatest inhibition of growth of total mesophilic microorganisms. The best results were obtained for a 30-second immersion time. Lettuce leaves were exposed to ozone for 60, 45 or 30 min. The 45-minute exposure time gave the best results.

The first batch of lettuce leaves was the control sample, and the second batch was immersed in a 1% w / v citric acid solution for 30 seconds. The third, fourth and fifth batches were treated with ozone gas at concentrations of 0.5, 1.0 or 1.5 ppm for 45 minutes and the last 3 batches were ozonated under the above conditions and immersed in 1% w / v citric acid solution for 30 seconds. All batches were packed in PET//LLDPE bags (20x25 cm, 12μm thickness for PET and 40μm for LLDPE) under passive modified atmosphere and stored under refrigeration for 12 days. Headspace analysis, color parameters (L*, a* and b*), pH, microbiological analysis (total mesophilic counts, yeast and molds counts and *Enterobacteriaceae* spp. counts) and sensory analysis were monitored every 3 days.

2.2. Headspace Analysis

A gas analyzer (PBI Dansensor 9900, Germany, Denmark) was used for the headspace analysis. Two bags from each treatment were measured by piercing with a hypodermic needle through an adhesive septum.

2.3. Color

A HunderLab model DP-9000 optical sensor colorimeter (Hunter Associates Laboratory, Reston, VA) was used to estimate color parameters: L* (lightness/ darkness), a* (redness/ greenness) and b* (yellowness/ blueness). For each treatment, four, 10-g, samples were placed in the cylindrical optical cell of the instrument. Reflectance values were obtained using a 45 mm viewing aperture. Five measurements per sample were performed.

2.4. pH

The pH was determined with a digital meter (Microprocessor pH Meter, HANNA Instruments, Romania). Ten g of sample from each treatment was homogenized with 100 mL of distilled water and filtered. The filtrate was used to determine pH. All experiments were carried out in duplicate and results expressed as the mean of the 2 measurements.

2.5. Microbiological Analysis

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Total mesophilic counts, yeast and molds counts and *Enterobacteriaceae* counts were measured during storage. Ten g of sample was weighed in a sterile stomacher bag, 90 mL of sterile peptone water (1% w/ v) was added and the mix homogenized for 2 min using a stomacher machine. Eight consecutive decimal dilutions of peptone water were prepared for each treatment. A 1 mL aliquot of the first decimal dilution was added to the second decimal dilution and vortexed. The same successive aliquot addition process was performed from each dilution to the subsequent one. For measurement of total mesophilic and yeast and mold counts, 100 μL of each dilution was transferred to the nutrient substrate contained in Petri dishes and spread using a sterile triangular glass rod. Plate count agar was used to enumerate total mesophilic counts and Rose Bengal Chloramphenicol to enumerate yeast and mold counts. Incubation conditions for total mesophilic and yeast and mold growth were 30^{o} C for 48 hours and 30^{o} C for 72 h, respectively. For enumeration of *Enterobacteriaceae*, 1 mL of each dilution was transferred to Petri dishes and nutrient substrate for growth of microorganisms Violet Red Brilliant Glucose agar was poured onto the inocula. After solidification of the nutrient substrate, Petri dishes were incubated at 37^{o} C for 24 hours. All samples were analyzed in duplicate and results expressed as the mean of the 2 measurements.

2.6. Sensory Analysis

A 7-member panel was used for the sensory analysis. Panelists were non-smokers and trained to distinguish small differences in sensory characteristics from between samples. Sensory characteristics estimated were general appearance, taste and odor. A 9-point scale was used to evaluate sensory characteristics as follows: 9 = excellent, 7 = very good, 5 = good, 3 = moderate, 1 = not acceptable. The limit of acceptability was set to 5.

2.7. Statistical Analysis

Results were tested for normality of distribution and homogenous variance and were subjected to two-way ANOVA using SPSS software for windows. If interactions were significant they were used to explain results. If interactions were not significant, means were separated using the least significance difference.

3. Results

3.1. Headspace Analysis

Results of headspace analysis are shown in Figures 1 and 2.

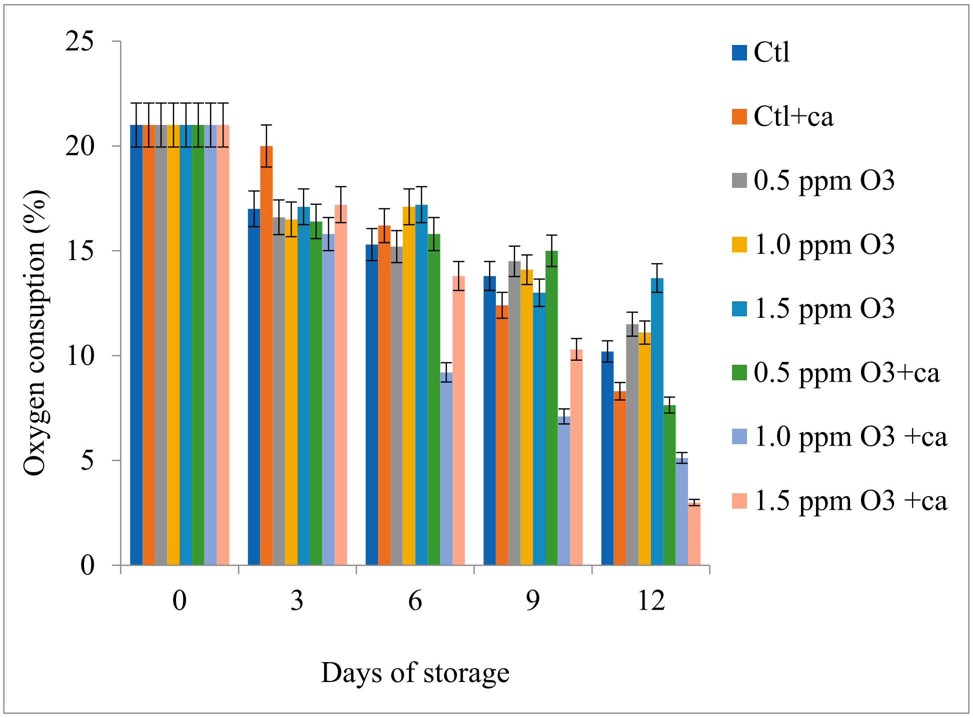

Figure 1. Effect of ozone and citric acid treatment on the rate of oxygen consumption of freshly cut lettuce during cold storage.

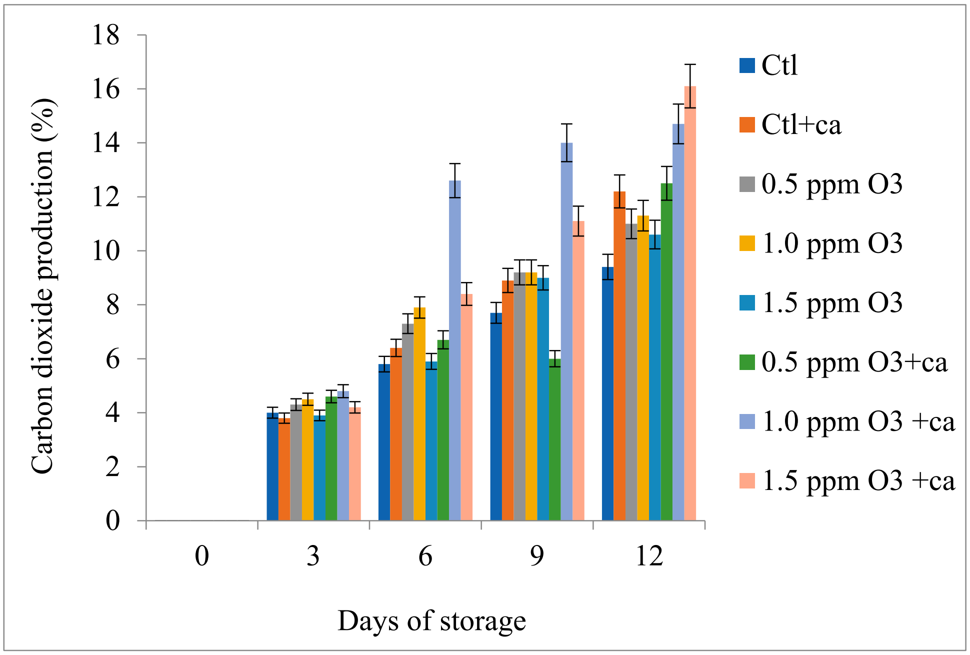

Figure 2. Effect of ozone and citric acid treatment on carbon dioxide production rate of freshly cut lettuce during cold storage.

Oxygen consumption rate in the sample bags decreased, while carbon dioxide production rate increased as a result of the respiratory activity of plant tissue. Ozone treatment did not affect the oxygen consumption rate significantly ($p>0.05$) compared to control sample. Oxygen consumption rate was affected significantly ($p<0.05$) in samples treated with ozone at 1.0 ppm plus citric acid compared to the control sample. A non-statistically significant ($p> 0.05$) higher oxygen consumption

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was observed in samples treated with ozone at 1.0 ppm plus citric acid and at 1.5 ppm plus citric acid compared to samples treated with only ozone at 1.0 and 1.5 ppm. The same response in carbon dioxide production was also observed. Carbon dioxide production rate was affected significantly ($p<0.05$) in samples treated with ozone at 0.5 and 1.0 ppm compared to control sample.

3.2. Color

The results of ozone and citric acid treatment on the color parameters are shown in Tables 1–3.

Table 1. Effect of ozone and citric acid treatment on color parameter L* of freshly cut lettuce during cold storage.

	Lettuce samples							
Days of storage	**Control**	**Control +citric acid**	**0.5 ppm ozone**	**1.0 ppm ozone**	**1.5 ppm ozone**	**0.5 ppm ozone +citric acid**	**1.0 ppm ozone +citric acid**	1.5 ppm ozone +citric acid
0	48.83 ^{1a} ±1.85	48.83 ^{3a} ±1.85	48.83 ^{4a} ±1.85	48.83 ^{5a} ±1.85	48.83 ^{8a} ±1.85	48.83 ^{9a} ±1.85	48.83 ^{11a} ±1.85	48.83 ^{12a} ±1.85
3	49.83 ^{1b} ±3.34	51.20 ^{3b} ±3.69	52.26 ^{4b} ±3.66	52.50 ^{6b} ±1.90	51.97 ^{8b} ±3.28	51.52 ^{9b} ±1.78	49.97 ^{11b} ±3.26	49.16 ^{12b} ±2.10
6	49,44 ^{1c} ±1.40	51.05 ^{3c} ±1.95	51.36 ^{4c} ±1.13	48.74 ^{7d} ±1.59	50.21 ^{8d} ±0.44	48.33 ^{10e} ±0.94	49.30 ^{11e} ±2.37	48.73 ^{12e} ±1.83
9	49,68 ^{1f} ±1.55	50.51 ^{3f} ±4.31	49.63 ^{4f} ±4.38	48.08 ^{7f} ±0.63	48.47 ^{8f} ±3.46	47.75 ^{10f} ±1.07	49.07 ^{11f} ±0.19	48.41 ^{12f} ±1.42
12	45.42 ^{2g} ±1.83	48.99 ^{3h} ±1.51	49.08 ^{4h} ±3.59	47.32 ^{7h} ±2.68	47.77 ^{8h} ±1.54	46.96 ^{10h} ±1.23	48.29 ^{11h} ±1.56	47.47 ^{12h} ±0.80

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

Table 2. Effect of ozone and citric acid treatment on color parameter a* of freshly cut lettuce during cold storage.

	Lettuce sample							
Days of storage	**Control**	**Control +citric acid**	**0.5 ppm ozone**	**1.0 ppm ozone**	**1.5 ppm ozone**	**0.5 ppm ozone +citric acid**	**1.0 ppm ozone +citric acid**	1.5 ppm ozone +citric acid
0	-16.18 ^{13i} ±0.53	-16.18 ^{14i} ±0.53	-16.18 ^{15i} ±0.53	-16.18 ^{16i} ±0.53	-16.18 ^{18i} ±0.53	-16.18 ^{19i} ±0.53	-16.18 ^{20i} ±0.53	-16.18 ^{22i} ±0.53
3	-16.78 ^{13j} ±0.28	-16.06 ^{14k} ±0.28	-16.37 ^{15k} ±1.01	-16.99 ^{16k} ±0.48	-16.58 ^{18k} ±0.48	-15.96 ^{19k} ±1.01	-16.85 ^{20k} ±0.22	-15.71 ^{22k} ±0.87
6	-16.58 ^{13l} ±0.57	-15.96 ^{14l} ±0.92	-16.18 ^{15l} ±0.35	-15.46 ^{17l} ±0.82	-15.61 ^{18l} ±1.25	-15.68 ^{19l} ±0.67	-15.78 ^{21l} ±0.66	-15.66 ^{22l} ±1.36
9	-15.28 ^{13m} ±1.82	-15.85 ^{14m} ±1.95	-15.64 ^{15m} ±0.99	-15.15 ^{17m} ±0.13	-15.52 ^{18m} ±0.74	-15.17 ^{19m} ±0.59	-15.25 ^{21m} ±1.16	-15.25 ^{22m} ±1.11
12	-14.93 ^{13n} ±1.52	-15.72 ^{14n} ±0.71	-15.51 ^{15n} ±1.16	-15.10 ^{17n} ±0.84	-13.99 ^{18n} ±2.28	-15.11 ^{19n} ±1.42	-14.40 ^{21n} ±0.88	-14.25 ^{22n} ±0.82

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

Table 3. Effect of ozone and citric acid treatment on color parameter b* of freshly cut lettuce during cold storage.

	Lettuce sample							
Days of storage	**control**	**Control +citric acid**	**0.5 ppm ozone**	**1.0 ppm ozone**	**1.5 ppm ozone**	**0.5 ppm ozone +citric acid**	**1.0 ppm ozone +citric acid**	1.5 ppm ozone +citric acid
0	35.33 [23o] ±1.87	35.33 [25o] ±1.87	35.33 [26o] ±1.87	35.33 [27o] ±1.87	35.33 [28o] ±1.87	35.33 [29o] ±1.87	35.33 [30o] ±1.87	35.33 [31o] ±1.87
3	37.86 [23p] ±1.31	36.99 [25p] ±1.16	36.75 [26p] ±2.06	37.74 [27p] ±1.95	37.46 [28p] ±2.07	35.18 [29p] ±2.09	37.82 [30p] ±0.75	35.24 [31q] ±1.45
6	37.57 [23r] ±0.71	35.81 [25s] ±1.05	36.43 [26s] ±0.98	35.68 [27s] ±0.65	35.66 [28s] ±3.90	35.10 [29s] ±0.87	35.79 [30s] ±2.13	34.80 [31s] ±2.73
9	33.95 [24t] ±1.73	35.79 [25t] ±2.28	35.15 [26t] ±1.01	33.84 [27t] ±1.47	33.74 [28t] ±2.08	34.84 [29t] ±1.04	35.34 [30t] ±1.99	34.64 [31t] ±1.83
12	33.86 [24u] ±3.70	35.50 [25u] ±1.59	34.14 [26u] ±1.61	32.96 [27u] ±1.83	33.11 [28u] ±4.16	33.99 [29u] ±1.36	31.96 [30u] ±2.47	32.83 [31u] ±2.15

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

A decrease in the L* parameter was observed in all treatments during cold storage due to the oxidation of phenolic compounds. L* parameter was not significantly affected ($p>0.05$) by treatments with ozone, citric acid and ozone plus citric acid compared to the control sample. The samples treated with ozone and ozone plus citric acid showed a higher L* value throughout the cold storage compared to the control sample.

In all treatments, an increase in the parameter a* was observed due to the degradation of chlorophyll and the oxidation of phenolic compounds. Treatment with ozone, citric acid and ozone plus citric acid did not significantly affect ($p>0.05$) a* parameter compared to control samples. A higher increase in a* parameter was observed in lettuce treated with 1.5 ppm ozone compared to the control sample. Lettuce treated with 0.5 ppm ozone showed a smaller increase in a* parameter.

A decrease in b * parameter was observed in all treatments because of the oxidation of carotenoids. Treatment with citric acid, ozone, and ozone plus citric acid did not significantly affect the parameter b * ($p> 0.05$) compared to the control sample. At the end of the storage period, the b * values of the ozone-treated samples at 1.0 ppm and 1.5 ppm were lower than those of the control sample. The same response was also observed in samples treated with ozone (concentrations of 1.0 and 1.5 nm) plus citric acid.

3.3. pH

The results of ozone and citric acid treatment on pH are shown in Figure 3.

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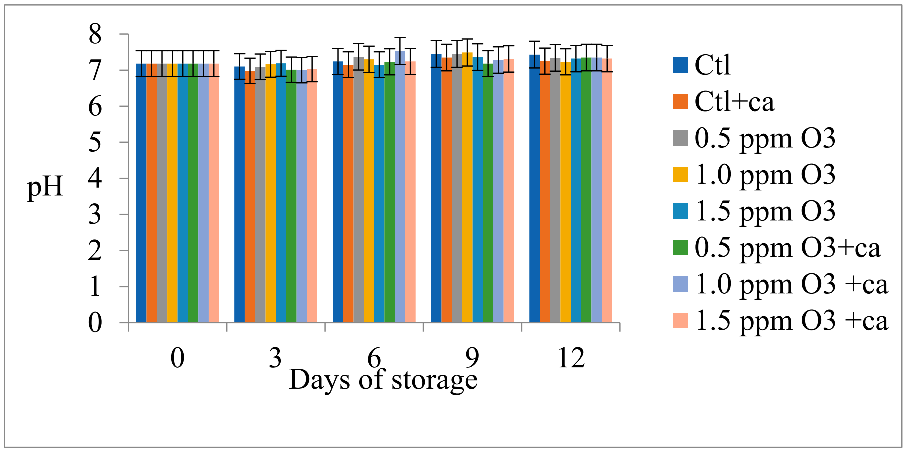

Figure 3. Effect of ozone and citric acid treatment on pH of freshly cut lettuce during cold storage.

A slight increase in pH was observed in the vast majority of treatments during cold storage. Treatment with citric acid significantly (pH <0.05) reduced the pH compared to the control sample. Ozone treatment and ozone treatment plus citric acid did not significantly (p>0.05) affect the pH compared to control sample.

3.4. Microbiological Analysis

The results of ozone and citric acid treatment on total mesophilic, yeast and molds and *Enterobacteriaceae* spp. counts are shown in Figures 4–6.

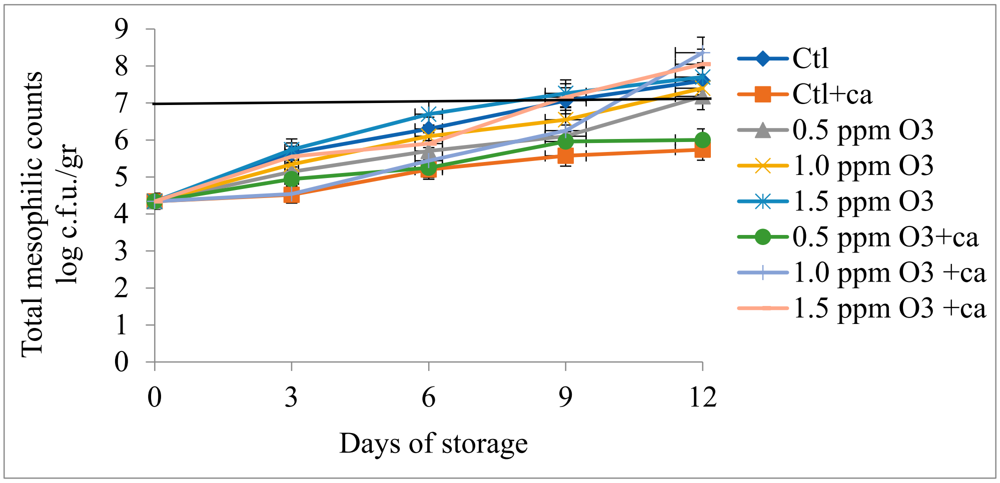

Figure 4. Effect of ozone and citric acid treatment on total mesophilic counts of freshly cut lettuce during cold storage.

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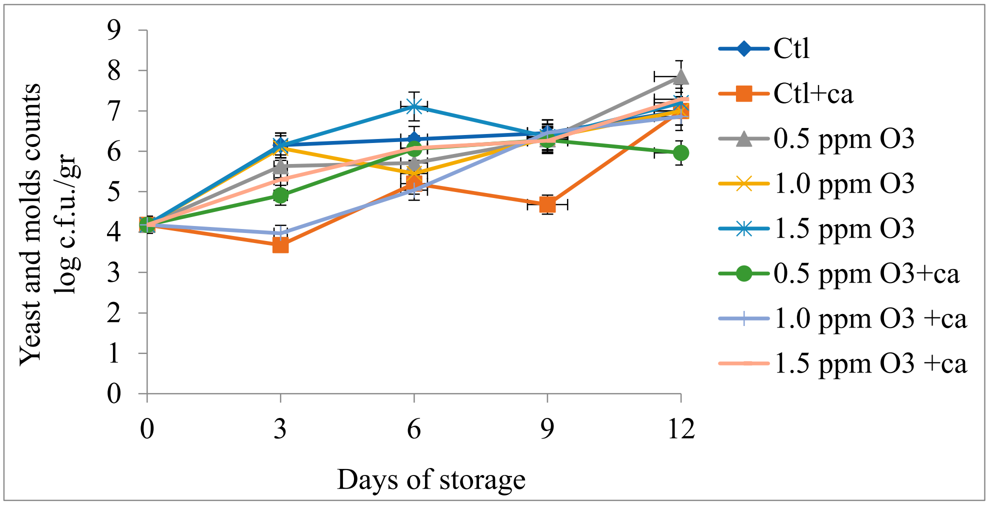

Figure 5. Effect of ozone and citric acid treatment on yeast and molds counts of freshly cut lettuce during cold storage.

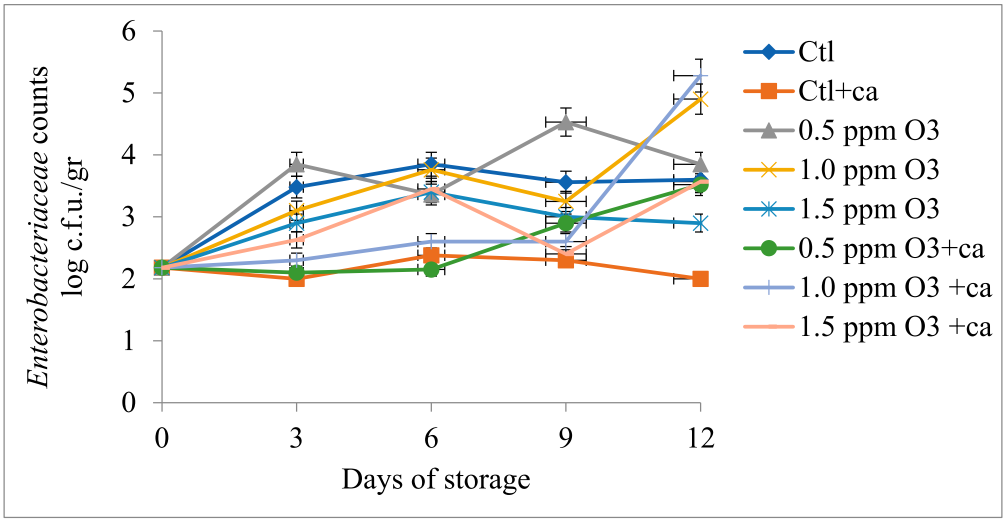

Figure 6. Effect of ozone and citric acid treatment on *Enterobacteriaceae* counts of freshly cut lettuce during cold storage.

Citric acid and ozone treatment at 0.5 and 1.0 ppm significantly reduced the population of total mesophilic counts ($p<0.05$), whereas ozone treatment at 1.5 ppm did not significantly ($p>0.05$) affect the total mesophilic counts compared to the control sample. In the control and ozone treated samples at 1.5 ppm and 1.5 ppm plus citric acid, total viable count levels exceeded the threshold limit value (7 $\log_{10}$c.f.u./g) on day 9 of storage. Total viable count levels in samples treated with citric acid and ozone at 0.5 ppm plus citric acid remained below the threshold limit until the last day of storage. In addition, samples treated with ozone at 0.5 ppm plus citric acid showed significant lower levels ($p<0.05$) of total mesophilic counts compared to the control samples. Irrespective of total mesophilic counts, treatment with ozone, citric acid and ozone plus citric acid did not significantly affect yeast and molds counts ($p>0.05$) compared to the control sample during storage.

Samples treated with citric acid showed a significantly ($p<0.05$) lower population of *Enterobacteriaceae* counts compared to the control sample. Ozone treatment at 0.5 and 1.0 ppm did not significantly affect ($p>0.05$) the *Enterobacteriaceae* counts compared to the control sample. On the 12th day of storage, the population of *Enterobacteriaceae* in samples treated with 0.5 and 1.0 ppm ozone was higher than that of the control sample. These ozone concentrations were insufficient to inactivate

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Enterobacteriaceae counts in freshly cut lettuce. On the other hand treatment with 1.5 ppm ozone significantly (p <0.05) reduced ozone *Enterobacteriaceae* counts compared to the control sample.

3.5. Sensory Analysis

The results of ozone and citric acid treatment on sensory analysis parameters are shown in Figures 7–9.

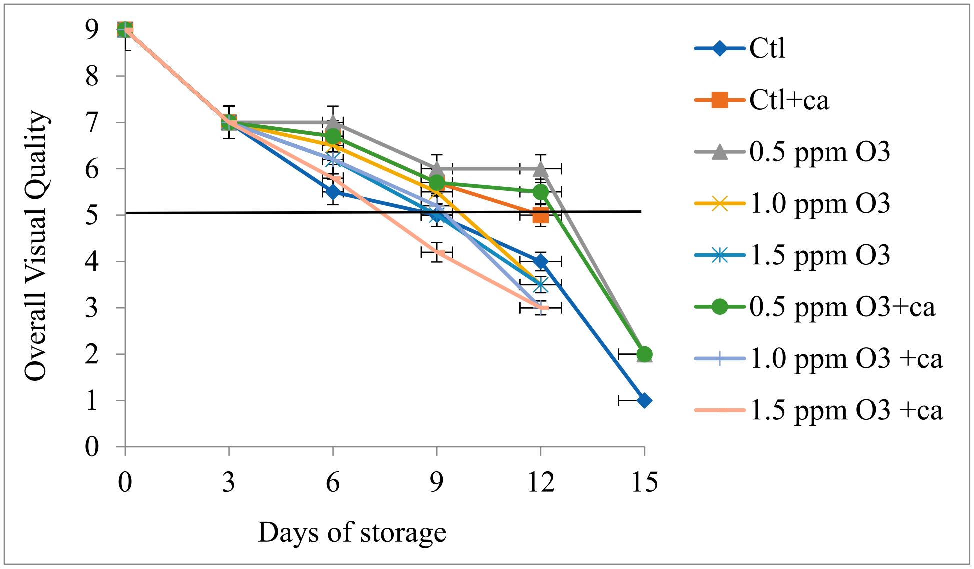

Figure 7. Effect of ozone and citric acid treatment on the appearance of freshly cut lettuce during cold storage.

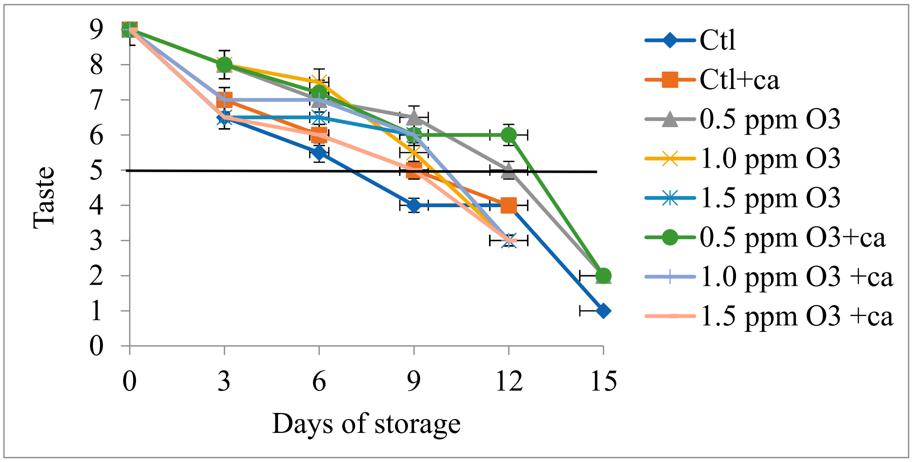

Figure 8. Effect of ozone and citric acid treatment on the taste of freshly cut lettuce during cold storage.

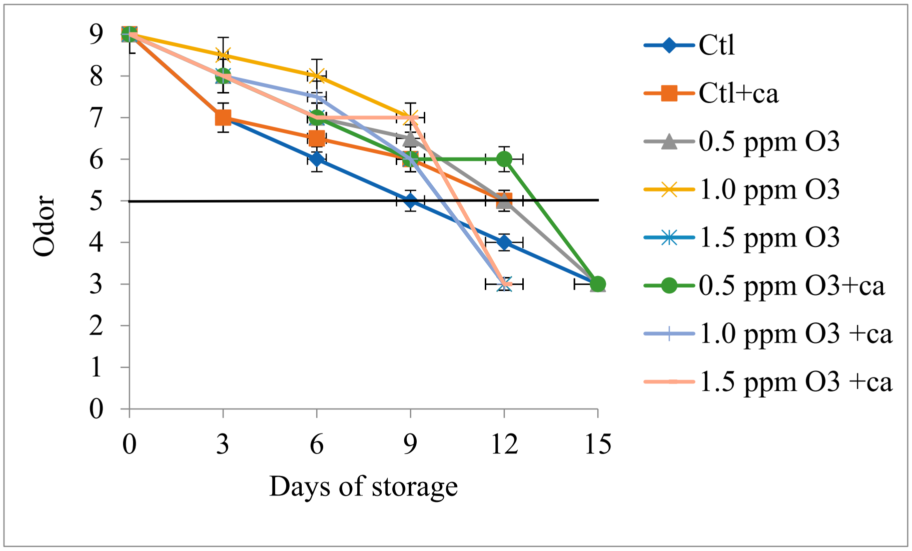

Figure 9. Effect of ozone and citric acid treatment on the odor of freshly cut lettuce during cold storage.

Cut edge vascular tissue browning (CEVTB) is the most serious defect of vascular midrib tissues responsible for reducing the overall visual quality (OVQ) of salad products. Ozone-treated samples at a concentration 0.5 ppm and 0.5 ppm plus citric acid showed lower CEVTB compared to the control sample. In contrast, samples treated with ozone at concentrations of 1.0 and 1.5 ppm and samples treated at concentrations of 1.0 and 1.5 ppm plus citric acid showed a higher CEVTB compared to the control sample as evidence of the high oxidizing capacity of ozone. Also, citric acid binds magnesium from the chlorophyll molecule and coverts it to brown-colored pheophytin. Obviously ozone treatment generally improves the sensory characteristics of fresh-cut lettuce throughout cold storage. The best results were observed with the treatment at 0.5 ppm. Ozonation at higher concentrations (1.0 and 1.5 ppm) results in faster degradation in the appearance of fresh-cut lettuce possibly due to the oxidizing action of ozone at high concentration.

4. Discussion

The application of ozone treatment did not cause a significant change in oxygen consumption rate in comparison with control sample during cold storage. Similar results were also reported by other investigators [14, 23, 24]. In contrast, ozone causes an increase in carbon dioxide production rate in comparison with untreated sample. All treatments including citric acid appeared a higher carbon dioxide production rate compare with untreated samples. This phenomenon is probably due to the oxidation of biomolecules of the cell membranes by ozone, causing an increase in oxygen leakage through the cell membrane. Citric acid also enters mitochondria and undergoes decarboxylation during the Krebs cycle. According to Rico et al. [25] the increase in respiration rate in ozone-treated fresh-cut lettuce could be attributed to the destruction of photosynthetic mechanisms that might alter the metabolism state.

Color parameters did not presented any significant change in comparison to control sample throughout of cold storage after the application of any treatment. In case of treatments with ozone and ozone plus citric acid the values of L* parameters were higher compared to control sample. This can be attributed to the inhibitory effect of citric acid on browning-related enzymes such as polyphenol oxidase and peroxidase [26, 27]. Goyeneche et al. [28] also found a higher L* value in sliced radish treated by immersion in citric acid solution compared to untreated radish. In contrast, Singh et al. [29] and Olmez and Akbas [14] found discolorations on ozone-treated lettuce. Moreover, ozone-treated spinach discoloration was reported by Vurma et al. [30] and Klockow and Keener [31]. Color parameters a* and b* was also not significantly affected after the application of all treatments.

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Also, Karaca and Velioglu [32] did not observe a significant change in chlorophyll content in ozone-treated parsley. Ozone treatment at 1.5 ppm causes a higher increment in a* parameter value compared to control sample, possibly due to the increased oxidizing capacity of ozone at high concentrations. In the ozone treated lettuces with 0.5 ppm the level of increase of a* parameter values were lower and this could be attributed to the inhibition of browning-related enzymes such as polyphenolic oxidase and peroxidase at low ozone concentrations [25]. Samples treated with citric acid showed a smaller increase in parameter a* compared to the control sample. There was no clear correlation between the measured color parameters and the sensory evaluation of OVQ and CEVTB and this lack of correlation could be attributed to the heterogeneity of the lettuce leaf mixtures [25, 33]. At the end of storage higher b* values were recorded in ozone-treated samples at 1.0 and 1.5 ppm, 1.0 ppm plus citric acid and 1.5 ppm plus citric acid. This could be attributed to the oxidizing effect of ozone on unsaturated compounds such as carotenoids.

The application of treatments did not cause significant reduction in pH value in comparison to untreated lettuces during cold storage, except of lettuces treated with citric acid. King et al. [34] and Jacxsens et al. [35] have claimed that the increase in pH is a result of the production of basic compounds due to the degradation of proteins by Gram-negative bacteria. Also, the application of other oxidizing compounds such as chlorine dioxide to lettuce and cabbage did not cause a significant change in pH values [36].

All treatments cause a reduction in total mesophilic counts compared to untreated samples during cold storage. Only lettuce treated with citric acid and 0.5 ppm plus citric acid retained their total mesophilic counts levels under the threshold limit value until the last day of storage. The above results are in general agreement with those of other researchers [37-39]. In contrast with total mesophilic counts the yeast and molds count did no present important changes after the application of treatments during cold storage. Gullen et al. [40] reported that yeast and molds are more ozone-resistant than bacteria due to their different membrane structure. In terms of Enterobacteriaceae counts, citric acid and ozone treatments at 1.5 ppm have shown the greatest reduction in Enterobacteriaceae population compared to the other treatments during cold storage. Beltran et al., [23] reported a 3.2 log reduction of coliforms in freshly cut ozonated water-treated lettuce compared to water-washed samples. Coliforms in freshly cut lettuce packaged in air and active MAP were lower by 4.2 log and 4.9 log, respectively, compared to water-washed lettuce on day 9 of storage. Kumar Das et al., [41] reported that treatment of fresh-cut iceberg lettuce with 3 μl/L ozonated water for 180 seconds reduced the Enterobacteriaceae count compared to tap water-washed samples. Baur et al. [33] also found that treatment of shredded iceberg lettuce with ozonated water at a concentration of 1 mg / L for 120 seconds resulted in a significant decrease in Enterobacteriaceae counts compared to tap water-washed samples. Other studies have shown that Enterobacteriaceae are generally sensitive to ozone treatment because they are Gram-negative bacteria and have thinner peptidoglycan layer on their cell wall compared to Gram-positive bacteria [42]. The sensitivity of bacteria to ozone treatment depends on factors such as ozone concentration and exposure time, the physical state of ozone, the ozone delivery method, temperature, pH, relative humidity, product type, number and type of microorganism, the location of microorganisms in the product and the physiological state of bacterial cell [12, 14, 26, 43]. In the present study we did not achieve a significantly higher rate of microbial inactivation using gaseous ozone. In contrast, in previous studies using bubbled ozonated water a higher rate of microbial inactivation was observed This can be explained by the so called "film theory". According to this theory, when ozone is bubbled into water, a liquid film is formed at the ozone-water interface, thereby increasing its concentration in the liquid film compared to that in the bulk liquid and in the gaseous phase [32].

Overall visual quality (OVQ) is a key feature used by consumers to assess the quality of fruits and vegetables [44-46]. In this indicator of quality estimation, lettuce treated with ozone at 0.5 ppm and 0.5 ppm plus citric acid possessed the lowest score in comparison with the untreated lettuces. Similar results were also found Garcia et al. [47], Beltran et al. [23], Akbas and Olmez [48] and Olmez and Akbas [14]. Some researchers attribute the ozone's ability to retain the quality characteristics of fresh-cut lettuce to its inhibitory effect on browning-related enzymes, such as polyphenoxidase and peroxidase [25].

5. Conclusions

Freshly cut lettuce samples treated with citric acid and ozone at 0.5 ppm plus citric acid showed significant lower total mesophilic counts compared to untreated samples. Both of the above treatments as well as the ozone treatment at 0.5 ppm showed improved sensory characteristics compared to untreated lettuce. Ozone treatment at 0.5 ppm and 0.5 ppm plus citric acid increased the shelf life of freshly harvested lettuce by 4 days under passive modified atmosphere packaging during cold storage, which positively contributes to the commercial value of the product. The shelf life extension achieved may at first sight appear rather small, but in our view is quite significant given the rapid wilting rate of broadleaf vegetables such as lettuce.

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