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Quality retention of minimally processed spinach using low-dose ozonated water during storage

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Abstract: The use of ozone for the sanitization of fresh-cut produce has been postulated during all steps of the cold chain from harvest to consumption. However, little is known about its effects on the postharvest quality of minimally processed spinach. In the present study, the effects of different doses of ozone on the storage life and quality of minimally processed spinach were determined and compared with sodium hypochlorite. Minimally processed spinach samples were subjected to 4 treatments: immersion in sodium hypochlorite solution (100 ppm) as control, and exposure to 3 different ozonated water (0.5,1 and 2 ppm) for 10 min. Treated spinach samples were packaged in modified atmosphere packaging and stored at 0 °C and 90 ± 5% relative humidity for 25 days. After each cold storage period, the spinach samples were kept for one day at 20 °C and 60 ± 5% relative humidity for shelf life studies. During cold storage and shelf life, weight loss, leaf color, SPAD value, respiration rate, gas composition in package, soluble solids content (SSC), and visual quality were determined. In general, weight losses of the spinach samples were delayed by ozone treatments, especially in low doses. The best suppressing dose for respiration rate of minimally processed spinach was 0.5 ppm, among all treatments. Additionally, the 0.5 ppm ozone treatment preserved vivid green color and visual quality of the spinach leaves better than those treated with higher doses and sodium hypochlorite. Although 1 and 2 ppm ozone treatments retarded quality losses by suppressing the respiration rates of spinach, some undesirable results were obtained from these doses due to their higher oxidative properties. The 0.5 ppm, therefore, can be considered as limit dose for postharvest treatments in minimally processed spinach. These findings revealed that ozonated water with appropriate doses can be used instead of sodium hypochlorite for minimally processed spinach during storage.

Key words: Cold storage, fresh-cut, ozone, quality loss, *Spinacia oleracea*

1. Introduction

The demand for healthy fresh fruit and vegetables has constantly been increasing due to their increasing trend in human diet. The share of minimally processed or fresh-cut vegetables, such as lettuce and spinach, due to this growing consumption, continues to increase day by day, especially in developed countries. The consumption of fresh spinach has reached record levels, increasing more than 12 times, in the last few decades in the USA (Klockow and Keener, 2009). Similarly, Gil et al. (1999) and Kaur et al. (2011) reported a huge demand for minimally processed spinach due to their utility in various recipes. The main reason for the increasing consumption of minimally processed vegetables is the fact that they are ready-to-use. Artes-Hernandes et al. (2009) indicated that minimally processed vegetables have gained enormous popularity due to their convenience, but this process provides an ideal medium for microbial development and causes tissue softening, off-odours, and discolouration. In particular, the main problems related to minimally processed spinach are off-odour, decay, and water and green color loss (Allende et al., 2004; Artes et al., 2007; Medina et al., 2012). The modified atmosphere packaging (MAP) was advised for maintaining the quality and safety of minimally processed spinach providing enough O_2 to prevent anaerobic conditions (Ko et al., 1996; Gil et al., 1999; Artes- Hernandes et al. 2009; Kaur et al., 2011). However, only MAP is not enough to improve the postharvest quality and extend storage life of minimally processed spinach because of increased metabolic activity and susceptibility to deterioration after the cutting process. Therefore, disinfection procedure with some agents is one of the most crucial stages to limit quality loss and microorganism growth during the processing of fresh-cut produce (Sapers, 2003).

Chlorine-based disinfectants, such as sodium hypochlorite, have been widely used for the sanitation of minimally processed vegetables in the last several decades (Cherry, 1999; Beltran et al., 2005). Consumers are becoming gradually concerned about the quality and

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safety of their food, because some carcinogenic products derived from the reaction of chlorine with organic residues affect human health and environmental safety (Parish et al., 2003; Beltran et al., 2005). Extending the postharvest life of horticultural crops by using chemicals is restricted due to their harmful effects on human health and the environment. Therefore, some research studies have focused on alternative agents, safety materials, and methods for prolonging the postharvest life of crops (Horvitz and Cantalejo, 2014). To prevent these harmful effects caused by chlorine-based disinfectants, environmentally friendly applications, such as ozone (O_3) and ultraviolet light (UV), should be deeply investigated.

Ozone, approved as a disinfectant agent (US-FDA, 2001), has attracted a lot of attention from scientists and people who work in different sectors (Karaca and Velioğlu, 2014). Used widely in food industry, ozone is a nontoxic and a strong antimicrobial agent (Whangchai et al., 2006). Ozone, applied as gas or ozonated water (Kuşçu and Pazır, 2004), has been tested for postharvest treatments in fruit and vegetables, and the positive effects of it have been studied by various researchers (Sarig et al., 1996; Palou et al., 2002; Dilmaçünal et al., 2014; Bayar Aydınoğlu et al., 2017; De Souza et al., 2018; Luo et al., 2019; Bolel et al., 2019a; Bülüç, 2019). Furthermore, ozonated water has been found to reduce quality losses and prolong the postharvest life of fresh-cut fruit and vegetables[1] (Sapers, 2003). Moreover, it has been reported that postharvest ozone treatments increase the resistance of plants, kill pathogen spores (Smilanick, et al., 1999), and decrease fungal deterioration (Whangchai et al., 2006) and microbial population (Zhang et al., 2005). Similarly, ozone treatments have extended the storage life and maintained quality of fresh cut celery (Zhang et al., 2005) and lettuce (Garcia et al., 2003) by reducing microbial population.

However, ozone treatment might cause physiological disorders and undesirable effects on the external appearance of leafy vegetables owing to its strong oxidizing activity (Horvath, 1985; Beltran et al., 2005). This disagreement between previous studies in terms of the effectiveness of ozone treatments can be explained by material variation (species and variety variation), application dose and time, and laboratory facilities, etc. There is a need to investigate the effect of different ozone doses and alternative treatment for sodium hypochlorite on postharvest quality and life of fresh fruit and vegetables. Therefore, sodium hypochlorite (100 ppm), which is widely used as a disinfectant in fresh-cut industry, was chosen as control group in the present study.

This study aimed to determine the effects of ozonated water with different doses on storage life and quality of minimally processed spinach in comparison with sodium hypochlorite.

2. Materials and methods

2.1. Plant material, minimally processing, and ozone treatments

The study was conducted with spinach (*Spinacia oleracea* L. cv. SV 1714 F1). The spinach samples, harvested at optimum time in the local orchard, were transferred to the laboratory by a refrigerated vehicle (4 °C) immediately (within 20 min) and precooled by forced air (2 °C). Foreign parts and injured plant materials were removed as well as yellow and withered leaves. After homogenization and visual examination, the spinach samples were washed with potable tap water to remove dirt and prepared for minimal processing. The roots and undesirable parts (outer leaves) of the spinach were removed byusing a sterile sharpened knife manually. Minimally processed spinach samples s were divided into 4lots. The first 3 groups were immersed in cold water (5 ± 1 °C) containing different concentrations (0.5, 1 and 2 ppm) of ozone for 10 min. The last group (control group) was immersed in cold water (5 °C) containing sodium hypochlorite at a concentration of 100 ppm, commercially used as disinfectant, for 9 min and then rinsed with potable tap water within 1 min (Lopez-Galvez et al., 2010). The solution of sodium hypochlorite (100 ppm available chlorine) was prepared using a concentrated solution (Tekkim Chemistry, Bursa, Turkey). The pH of solution (6.5) was adjusted by hydrochloric acid.

Ozone gas was generated by using a lab-scale oxygen-fed ozone generator (corona discharge, Ozonoks System-Model: CFY20, Antalya, Turkey) with a capacity of 20 g h^{-1}.Ozone gas was dissolved in cold water using a system containing a water pump, a micro bubble apparatus, and a contact tank (Figure). The dissolved ozone concentration in water was measured automatically by a sensor (Ozone Sensor, OZ7MA5, Dosatronic GmbH, Ravensburg, German) mounted on the ozone generator. The gas flow was controlled automatically by the control unit (JUMO-AQUIS 500, JUMO GmbH & Co. KG., Fulda, German) of the ozone generator during treatments. The temperature of the treatment room was set to 5 °C.

2.2. Packaging and storage

Treated spinach samples were dried under a ventilator at room condition (20 °C) for 15 min. After drying, the spinach was packaged (each containing 750 g samples) in modified atmosphere bags (LifePack-LDPE) and stored at 0 °C and 90 ± 5% relative humidity (RH) for 25 days. All treatments and packaging procedures were carried out under sanitary conditions in the laboratory. After

[1] Beuchat LR (1998). Surface decontamination of fruits and vegetables eaten raw: a review. World Health Organization Food Safety Unit, WHO/FSF/FOS/98.2 [online]. Website http://www.who.int/fsf/fos982-1.pdf [accessed 13 January 2020].

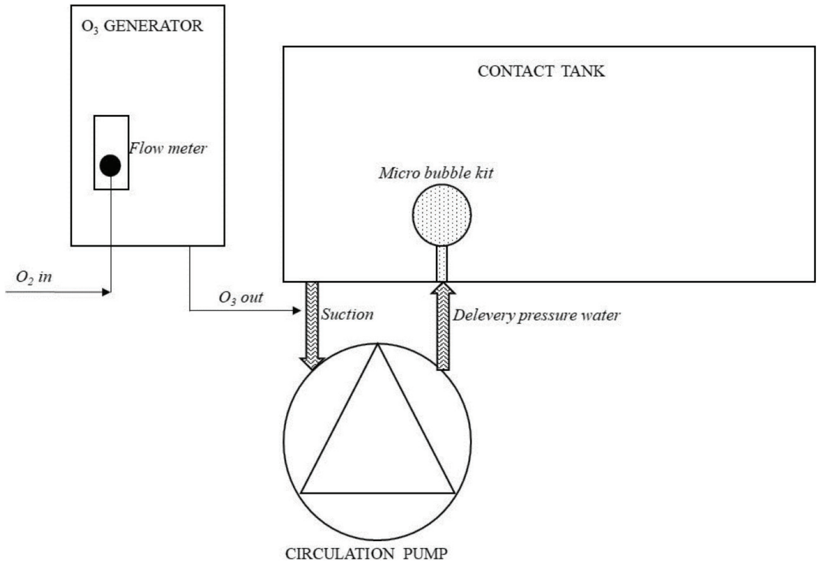

Figure. Ozonation of water using an ozone dissolving system combined with ozone generator.

cold storage, spinach samples were kept at 20 °C and 60 ± 5% RH for 1 day for shelf life evaluation. The following chemical and physical analyses were performed during cold storage and shelf life.

2.3. Chemical and physical analysis

Weight loss: Weight loss of spinach was measured based on the initial weight and calculated as percentage (%) during cold storage. The weight of each sample group was measured each analysis day (0, 5, 10, 15, 20, and 25) at the end of cold storage and shelf life sampling days (+1 day). Weight loss during shelf life was calculated from the difference between the initial and final sample weight as percentage (%).

Soluble solids content (SSC): The SSC of spinach juice was determined with a refractometer (Digital-Atago Pocket PAL-1) and expressed as percentage (%).

Leaf color: Leaf color was measured with a colorimeter (Minolta CR 300, Konica Minolta Business Solutions U.S.A., Inc., Ramsey, NJ, USA). The calibration of color measurement apparatus (Minolta) was performed using an original calibration plate (white). The values were evaluated according to CIE L* (represents brightness-darkness changing from 0 to 100), C* (represents vividity of color), and h° (represents perceived color) system. The chroma (C*) and hue angle (h°) values were calculated using the following formulas: $h° = \tan^{-1} (b^*/ a^*)$, $C^* = [(a^*)^2 + (b^*)^2]^{1/2}$.

SPAD value: The SPAD (soil plant analysis development) value (the way of estimating the leaf chlorophyll concentration by nondestructively) was measured with a SPAD meter (Minolta 502, Minolta Camera Co., Ltd., Osaka, Japan). It represents the green color intensity of leaves. Color and SPAD value measurements were performed on 3 random points of 10 spinach leaves (total 30 measurements for each replicate), and the average values of 30 measurements were used.

Respiration rate: Respiration rate was determined using a sample of 75 ± 5 g. The samples were weighed in 0.5 L airtight jars at room condition (20 °C). After 2 h, the gas sample was taken from the jars using a gastight syringe and injected into loop of gas chromatography (GC). Gas measurements were performed in split/splitless (S/SL) of inlet in split mode with valve and fused silica capilar column (GS-GASPRO, 30 m × 0.32 mm I.D., USA). Respiration rate was measured by Agilent model (6890N) GC using thermal conductivity detector (TCD). The carrier gas flow was 1.7 mL min^{-1}. The temperature of the oven was chosen as 40 °C (isothermal). The temperature of the TCD was 250 °C. Results were calculated as mL $CO_2 kg^{-1} h^{-1}$(Lemoine et al., 2007).

Gas composition of modified atmosphere package (MAP*):* Gas concentration (O_2 and CO_2) in the packages was measured by Gaspace 2 (Gas Headspace analyzer, Systech Instruments Ltd., Oxford, UK), and expressed as percentage (%).

Sensory analysis: The sensory evaluation panel consisted of 7 members of the research staff (Horticulture Department) who were experienced in sensory analysis of horticultural crops. Spinach samples (coded with 3-digit numbers) were served at room temperature and analysed under fluorescent light in a sensory evaluation room. External appearance (visual quality) was regarded a key sensorial characteristic for minimally processed spinach by panellists. The hedonic scale was used for the evolution of external appearance of spinach samples:

External appearance (scale 1–9): poor quality: 1–4; marketable quality: ≥ 5; good quality: 7–8; excellent quality: 9.

2.4. Statistical analysis

The completely randomized design was chosen for the experiment. Three replications, each containing 750 g samples of each experiment, were carried out. Using software package (SPSS v.18.0, SPSS Inc., Chicago, IL, USA), the general linear model (GLM) was used for statistical analyses. The differences among means (at a significance level of 0.05) were analysed using Tukey test.

3. Results and Discussion

3.1. Weight loss

The different doses of ozone and storage time significantly affected the weight loss of the minimally processed spinach during cold storage (Table 1). As expected, weight losses of all treated spinach increased with the increasing storage time. However, ozone treatments, depending on doses, delayed these increases compared to the control group. The lowest average weight losses (0.19% and 10.53%) were obtained from spinach treated with 0.5 ppm ozone in cold storage and room condition, respectively. The higher doses than 0.5 ppm, especially 2 ppm, gave similar weight losses (0.34% and 13.33%) to control groups (0.38% and 13.04%) during cold storage and shelf life (Tables 1 and 2). These results show that 0.5 ppm ozone may be a threshold value for weight loss in minimally processed spinach because of the negative effects of higher doses. As it is known, the weight loss of fresh fruit and vegetables is affected by transpirationfrom product surface,respiration rate, and electrolyte leakage. In the present study, it is thought that 1 and 2 ppm ozone doses increased the surface permeability of spinach leaves by oxidizing succulent skin. High ozone doses, with strong oxidizing effect, might have increased stoma permeability by degenerating the surface of the leaves. In previous studies, the effects of ozone treatments on weight loss were different depending on dose, application time and type, variety or species of crop, and storage conditions. Tabakoğlu and Karaca (2018) and Bülüc (2019) found that weight losses of fruit and vegetables decreased with the increasing ozone concentration during storage. Similarly, Çakır et al. (2014) and Bolel et al. (2019a) reported that ozone treatment decreased the weight loss of different horticultural crops during cold storage. On the other hand, weight loss of ozone treated fruit was higher (Cayuela et al., 2009) or did not change (Palou et al., 2002) when compared to the control groups. The weight loss of leafy vegetables is a crucial and commercial parameter. It should be taken into consideration during storage, because the weight loss in leafy vegetables is directly related to the decrease in visual quality and product weight.

3.2. Respiration rate

The effects of ozone doses and storage time on respiration rate were significant both during cold storage and room condition (Tables 1 and 2). The respiration rates of samples fluctuated during storage, but decreased at the end of the storage compared to the initial values in both conditions. As observed in the weight loss values, 0.5 ppm was the best suppressing dose for the respiration rate of spinach. The lowest value (17.2 $mLCO_2$ $kg^{-1}h^{-1}$) was measured in 0.5 ppm treatment followed by 1 ppm (18.6 $mLCO_2$ $kg^{-1}h^{-1}$), 2 ppm (21.0 $mLCO_2$ $kg^{-1}h^{-1}$), and control group (22.2 $mLCO_2$ $kg^{-1}h^{-1}$). A similar trend was also observed in ambient storage (Table 2). The increase in respiration rates depending on increasing ozone doses can be explained by the increased stress response of the spinach to high ozone concentration (1–2 ppm). It is thought that high doses of ozone might have stimulated stress response in the spinach, and thus increased respiration. On the other hand, increased gas permeability of leaves with higher doses, as mentioned in the weight loss section, can be the reason for higher respiration rates. In the present study, the suppressing effect of 0.5 ppm ozone on respiration rate is in accordance with the findings of the previous researchers who reported that ozone treatments decreased respiration rates by delaying metabolic activity and senescence processes in different horticultural crops during storage (Zhang et al., 2005; Bolel et al., 2019a, 2019b). The positive effect of ozone on respiration rate can also beattributed to its ability to increase systemic resistance in crops by increasing antioxidant capacity, which may suppress respiration processes in cells (Artes-Hernandez et al., 2007). On the contrary, Beltran et al. (2005) reported no significant differences between ozonated fresh-cut lettuce and control sample in terms of respiration rate. Therefore, the choice of ozone dose and treatment duration that induce an additional stress on crops for some physiological processes is crucial.

3.3. Gas composition of MAP

The O_2 and CO_2 concentrations of MAP were statistically affected by storage time and treatments during cold storage (Table 1). The gas composition in bags of minimally processed spinach changed during cold storage. The initial O_2 content (21 ± 0.1%) of bags decreased to 16.8% on the 5th day of storage and changed between 17.5% and 20.9% in the rest of the storage period. The average initial CO_2 concentration increased and reached a peak value of 3.5% in the first 5 days of storage, and then decreased contrarily to O_2 content in the last 20 days (Table 1). This changing tendency in both O_2 and CO_2 indicates that continuous stability of gases in package is not established well. Nevertheless, the CO_2 dose, changing between 1.5–2.0%, can be considered enough for suppressing some metabolic processes related to senescence. While 0.5

Table 1. Some quality parameters (weight loss, respiration rate, leaf color, soluble solids content, external appearance, SPAD value, and gas composition of MAP) of minimally processed spinach dipped in ozonated (0.5, 1, 2 ppm) and chlorinated (control-100 ppm) water during cold storage.

SD	T	WL	RR	L*	C*	h°	SSC	EE	SPAD	$\%O_2$	$\%CO_2$
0	Control	-	16.1	41.7	27.3	125.6	7.4c–f	9.0	57.0	21.0a	0.03k
	0.5 ppm	-	16.1	41.7	27.3	125.6	7.4c–f	9.0	57.0	21.0a	0.03k
	1 ppm	-	16.1	41.7	27.3	125.6	7.4c–f	9.0	57.0	21.0a	0.03k
	2 ppm	-	16.1	41.7	27.3	125.6	7.4c–f	9.0	57.0	21.0a	0.03k
5	Control	0.13	30.1	36.4	26.3	124.6	8.2a–d	9.0	53.7	16.8f	3.5a
	0.5 ppm	0.04	24.6	34.3	27.1	125.3	7.6b–e	9.0	55.7	17.8de	2.4b–d
	1 ppm	0.13	22.0	32.6	25.0	125.2	6.4g	9.0	55.5	17.6e	2.7b
	2 ppm	0.14	28.9	34.5	26.6	124.8	7.3d–f	9.0	52.4	17.8de	2.4b–d
10	Control	0.23	31.8	42.1	26.1	127.9	9.1a	8.1	44.3	17.5e	2.6bc
	0.5 ppm	0.11	26.8	42.4	25.1	127.8	7.0e–g	8.5	46.9	18.1de	2.0d–g
	1 ppm	0.23	27.7	43.5	30.0	124.7	7.4c–f	7.7	47.1	18.0de	1.9e–g
	2 ppm	0.23	32.4	46.3	29.9	123.4	7.4c–f	8.0	47.8	17.9de	2.2c–f
15	Control	0.33	29.7	32.5	25.9	123.1	8.5ab	6.3	47.4	17.8de	2.2c–f
	0.5 ppm	0.19	20.8	40.4	27.4	122.9	7.4c–f	7.2	53.7	18.3d	1.6g–j
	1 ppm	0.33	24.9	38.1	27.5	124.1	6.8e–g	6.0	45.0	17.8de	2.0d–g
	2 ppm	0.34	28.0	34.4	26.3	123.4	6.9e–g	4.9	47.1	18.0de	1.8f–h
20	Control	0.44	11.3	42.6	29.9	122.6	8.5ab	4.3	39.7	19.9c	2.1d–g
	0.5 ppm	0.27	4.4	40.3	28.0	125.5	6.9e–g	5.0	42.3	20.9a	1.4h–j
	1 ppm	0.44	8.6	42.4	29.1	123.4	7.6b–f	4.7	45.0	20.3bc	1.8f–h
	2 ppm	0.44	8.6	45.0	30.1	123.1	7.4c–f	4.7	48.4	20.9a	1.3ıj
25	Control	0.76	14.2	46.8	28.1	121.3	8.3a–c	2.8	42.7	20.7ab	1.8f–ı
	0.5 ppm	0.34	10.8	43.8	29.6	123.4	7.0e–g	3.1	45.3	20.9a	1.2j
	1 ppm	0.54	12.1	45.2	29.4	123.8	6.7fg	2.9	46.0	20.9a	1.3ıj
	2 ppm	0.57	12.2	46.4	30.8	121.8	6.9e–g	2.9	45.2	20.9a	1.2j
T means	Control	0.38a	22.2a	40.3	27.3	123.6b	8.3a	6.6	47.5	19.0c	2.0a
	0.5 ppm	0.19b	17.2b	40.5	27.4	125.1a	7.2b	7.0	50.1	19.5a	1.5c
	1 ppm	0.33a	18.6b	40.6	28.1	124.5ab	7.0b	6.6	49.3	19.3b	1.6b
	2 ppm	0.34a	21.0ab	41.4	28.5	123.7b	7.1b	6.4	49.7	19.4a	1.5c
SD means	0	-	16.1c	41.7a	25.5bc	125.6a	7.4ab	9.0a	57.0a	21.0a	0.03f
	5	0.11d	26.4ab	34.4b	29.2c	125.0ab	7.4ab	9.0a	54.3ab	17.5d	2.7a
	10	0.20cd	29.9a	43.6a	29.9a–c	125.9ab	7.7a	8.1b	46.5c	17.9c	2.2b
	15	0.30bc	25.9b	36.4b	28.6bc	123.4bc	7.4ab	6.1c	48.3bc	18.0c	1.9c
	20	0.40b	8.2d	42.6a	29.5ab	123.7a–c	7.6a	4.7d	43.8c	20.5b	1.7d
	25	0.55a	12.3c	45.6a	28.4a	122.6c	7.2b	3.0e	44.8c	20.9a	1.4e
P-values											
SD		**	**	**	**	**	**	**	**	**	**
T		**	**	NS	NS	*	**	NS	NS	**	**
SD × T		NS	NS	NS	NS	NS	**	NS	NS	**	**

SD: Storage days; T: Treatments; WL: Weight loss (%); RR: Respiration rate ($mLCO_2 kg^{-1} h^{-1}$); C*: Chroma; h°: Hue angle SSC: Soluble solids content (%); EE: External appearance (1–9 score); SPAD; Soil plant analysis development; NS represents nonsignificance at $P < 0.05$; ** Represents significance at the 0.01 level; * Represents significance at the 0.05 level. Means followed by different letters within the same column are significantly different ($P < 0.05$).

Table 2. Some quality parameters (weight loss, respiration rate, leaf color, soluble solids content, external appearance, and SPAD value) of minimally processed spinach dipped in ozonated (0.5, 1, 2 ppm) and chlorinated (control-100 ppm) water during shelf life.

SD	T	WL	RR	L*	C*	h°	SSC	EE	SPAD
0+1	Control	13.9b–g	29.9a–c	37.4	25.2	125.4a	8.3h–j	9.0	48.8
	0.5 ppm	11.5d–i	21.6c–g	35.7	23.5	126.5a	9.9c–e	9.0	48.4
	1 ppm	12.2c–h	17.2f–i	38.3	26.8	124.9ab	9.2e–g	9.0	47.6
	2 ppm	12.4b–h	24.4b–f	39.4	26.3	125.6a	7.4k–m	9.0	47.4
5+1	Control	7.8e–i	31.8ab	43.3	30.2	123.5ab	13.8a	8.9	39.2
	0.5 ppm	8.6e–i	26.8a–e	39.4	26.2	126.4a	11.4b	9.0	44.6
	1 ppm	14.1b–f	27.7a–d	43.1	30.4	123.2ab	11.2b	9.0	42.2
	2 ppm	10.3d–i	34.3a	43.9	29.8	124.3ab	11.1b	8.9	40.4
10+1	Control	4.2hi	16.8f–i	42.7	29.5	125.2a	9.5d–f	7.7	43.8
	0.5 ppm	4.5g–i	14.0g–i	44.8	29.9	124.7ab	8.1h–k	7.2	43.8
	1 ppm	2.3i	13.8g–i	42.8	28.0	126.3a	7.4k–m	7.6	42.2
	2 ppm	3.9hi	22.0c–g	46.7	32.2	122.8ab	8.4g–i	7.3	42.4
15+1	Control	25.6a	23.4b–f	42.4	27.5	124.6ab	10.6bc	5.4	37.7
	0.5 ppm	21.3a–c	18.5e–h	38.5	26.8	124.0ab	8.9f–h	6.2	42.2
	1 ppm	18.9a–d	13.0g–i	44.5	31.3	123.6ab	8.5g–i	5.9	40.3
	2 ppm	24.8a	19.4d–h	40.2	28.6	124.3ab	9.9c–e	4.6	40.5
20+1	Control	21.8ab	19.8d–h	49.6	33.2	119.1b	10.1cd	2.8	46.0
	0.5 ppm	8.9e–i	11.3hi	39.1	26.3	125.7a	7.8i–l	3.3	47.5
	1 ppm	18.9a–d	17.1f–i	41.8	28.3	124.6ab	7.3lm	2.9	45.3
	2 ppm	14.9b–e	11.8hi	44.9	30.2	123.9ab	7.1lm	3.0	48.5
25+1	Control	5.0f–i	8.2i	44.2	29.5	122.5ab	7.6j–m	2.0	45.2
	0.5 ppm	8.4e–i	9.5i	39.4	26.0	125.4a	6.9m	2.9	41.3
	1 ppm	6.5e–i	13.5g–i	42.7	28.4	124.0ab	7.0lm	2.9	40.4
	2 ppm	10.6d–i	14.4g–i	46.2	29.8	121.4ab	7.0lm	2.7	41.4
T means	Control	13.0	21.6a	43.3a	29.2a	123.4b	10.0a	6.0	43.5
	0.5 ppm	10.5	17.0b	39.5b	26.5b	125.5a	8.8b	6.3	44.6
	1 ppm	12.2	17.1b	42.2ab	28.9a	124.4ab	8.4c	6.2	43.0
	2 ppm	13.3	21.1a	43.6a	29.5a	123.7b	8.5c	5.9	43.4
SD means	0+1	12.5bc	23.3b	37.7b	25.5b	125.6a	8.7c	9.0a	48.1a
	5+1	10.2cd	30.2a	42.4a	29.2a	124.3ab	11.9a	9.0a	41.6c
	10+1	3.8e	16.7cd	44.2a	29.9a	124.7ab	8.3d	7.4b	43.1bc
	15+1	22.7a	18.6c	41.4ab	28.6ab	124.1ab	9.5b	5.5c	40.2c
	20+1	16.1b	15.0d	43.8a	29.5a	123.4b	8.1d	3.0d	46.9ab
	25+1	7.6d	11.4e	43.1a	28.4ab	123.3b	7.1e	2.6d	42.0c
P-values									
SD		**	**	**	**	*	**	**	**
T		NS	**	**	**	**	**	NS	NS
SD × T		**	**	NS	NS	*	**	NS	NS

SD: Storage days; T: Treatments; WL: Weight loss (%); RR: Respiration rate (mLCO_2kg^{-1}h^{-1}); C*: Chroma; h°: Hue angle SSC: Soluble solids content (%); EE: External appearance (1–9 score); SPAD; Soil plant analysis development; NS represents nonsignificance at $P < 0.05$; ** Represents significance at the 0.01 level; * Represents significance at the 0.05 level. Means followed by different letters within the same column are significantly different ($P < 0.05$).

ppm ozone treatment gave the lowest CO_2 (1.5%) and the highest O_2 ratios (19.5%), opposite values (CO_2 = 2.0%; O_2 = 19.0%) were obtained from the bag containing control samples. These resultsindicated that 0.5 ppm was the best dose suppressing respiration rate of spinach, compared to control and other ozone doses. As can be seen in Tables 1 and 2, the higher doses (1 and 2 ppm) also decreased respiration rates better than control group. Respiration rate results confirm the findings related to gas compositions in MAP.

The CO_2 contents of MAP increased, while O_2 concentrations decreased, according to the initial values (21%; 0.03%), depending on the permeability of the package material and respiration rate of minimally processed spinach, in all treatments during storage. Similar results were obtained from previous studies carried out by Lopez-Galvez et al. (2010), Bolel et al. (2019a), and Koyuncu et al. (2018) in different fruit and vegetables. On the other hand, there were no differences in the gas concentrations of fresh-cut vegetable packages regardless of treatments, such as sodium hypochlorite, ozone, and tap water (Baur et al., 2004; Allende et al., 2008; Lopez-Galvez et al., 2010). This can be due to different species, doses, and application types. Similar to our findings, Yeşilçimen Akbaş and Ölmez (2007) reported an increase in CO_2 and a decrease in O_2contents in ozone-treated lettuce during storage. However, the changing of gas compositions (O_2= 0% and CO_2= 15% on the 10th day of storage) in package was dramatic compared to our data. These findings revealed that the permeability of modified atmosphere bags used in the present study was higher and more appropriate than that of the previous study carried out by Yeşilçimen Akbaş and Ölmez (2007).

3.4. Leaf color

The effects of both storage time and treatments on L* and C* values were only significant during shelf life (Table 2). However, hue angle was affected by storage time and treatments during cold storage and room condition. Color changes (L*, C*, h°) of minimally processed spinach during storage are presented in Tables 1 and 2. In leaf-edible vegetables, leaf color is considered one of the most important quality parameters because it affects the commercial value of crops. The L* values of leaves fluctuated during storage and increased at the end of storage compared to the initial values both in cold room and ambient condition. There was not much variation between ozone-treated and untreated samples for L* values in cold storage.In general, L* values in ambient condition were higher than those in cold room condition. Increased L* values in room condition can be explained by the decrease in green color intensity with senescence, and therefore the increase in whiteness. The highest L* values were obtained from 2 ppm ozone treated leaves (41.4) while the control sample gave the lowest one (40.3) in cold storage. This trend, however, was not observed in shelf life study. These results are similar to those reported by Sengun and Kendirci (2018), who indicated that ozone treatment in water did not cause any differences in the L* value of minimally processed lettuce. Similar findings were also reported by Glowacz et al. 2015a and 2015b in different horticultural crops. On the other hand, after 12 days of storage, a significant decrease in L* values of lettuce dipped in ozonated water (4 ppm) was observed as compared with the initial values (Yeşilçimen Akbaş and Ölmez, 2007).

The C* values tended to rise with the increasing storage period in all treatments during cold storage as well as shelf life period. A similar trend was also observed by Papachristodoulou et al. (2007) and Uner (2018) in ozonated fresh cut spinach and parsley throughout storage. The average C* values, which express the vividity, changed dose-dependent in cold storage, but this tendency was not obvious in ambient condition. The highest C* values (28.5 and 29.5) were obtained from spinach treated with 2 ppm in both conditions. This can be explained by the brightness effect of high dose ozone on the surface of spinach leaves. In accordance with our data, Papachristodoulou et al. (2007) and Sengun and Kendirci (2018) reported that the C* values of the minimally processed spinach and lettuce were not statistically affected by ozone treatment. Similarly, low dose ozone (0.7 µmol mol^{-1}) exposure to the minimally processed peppers had no effect on skin colour (Horvitz and Cantalejo, 2012).

The h° angle values, which represent the perceived color, show the change from yellow to green as the angle value increases after 90°. In the present study, at harvest, the color of the spinach leaves was dark green corresponding to a hue angle (h°) value of 125.6°. This value decreased with increasing storage time in both cold room and ambient conditions depending on increasing yellowness on the surface of the leaves. The highest h° values, which represent dark green in spinach, were obtained from 0.5 ppm ozone treated leaves (125.1° and 125.5°) in cold storage and room condition, respectively, while the lowest ones (123.6°; 123.4°) were determined in control samples followed by 2 ppm dose (123.7°; 123.7°). These results showed that 0.5 ppm ozone treatment maintained green color, with minimum change in h° values, in minimally processed spinach leaves when compared to the control and other ozone doses. The negative effect of high doses, especially 2 ppm, can be attributed to its oxidizing (chlorophyll degradation) properties. Similar to our data, Papachristodoulou et al. (2007) found that ozone treatment (0.8 ppm) in water gave higher h° values in minimally processed spinach at the end of cold storage. The positive results obtained from low (0.5 ppm) dose, in the present study, are in accordance with previous research studies (Papachristodoulou et al., 2007; Horvitz and Cantalejo, 2012; Glowacz et al., 2015b) in which low doses of ozone did not bleach the pigments on the skin of spinach and peppers.

3.5. SPAD value

No significant differences in SPAD values were observed among treatments. However, the effect of storage duration on SPAD values was significant. The SPAD values, which represent the green colour intensity of leaves, decreased throughout the cold storage and shelf life (Tables 1 and 2). All the samples treated with ozone had higher SPAD values than those immersed in water containing sodium hypochlorite (control). The highest value (50.1) was obtained from 0.5 ppm ozone treated spinach followed by 2 ppm (49.7), 1 ppm (49.3), and control (47.5) in cold storage. The differences in SPAD values between ozonated leaves and control group during cold storage disappeared when the spinach samples were transferred to room condition. The SPAD values of all the samples stored in ambient condition were lower than those in cold storage. This can be explained by the increase in senescence-associated processes and the decrease in chlorophyll contentin leaves depending on high atmosphere temperature. It is well known that the chlorophyll content of leafy vegetables decreases with increased storage time, especially in ambient conditions. In fact, some previous studies showed that increase in storage time resulted ina decrease in the chlorophyll content of minimally processed spinach (Lisiewska et al., 1997; Cefola and Pace, 2015; Oliveira et al., 2016). Degradation of chlorophyll pigment in spinach leaves appeared to be regulated through the pathway of peroxidase-hydrogen peroxide, thus resulting in a colorless compound (Yamauchi and Watada, 1991). Similarly, Karaca and Velioğlu (2014) reported that the chlorophyll content of ozone treated fresh-cut spinach was higher (15.30 g kg^{-1}) than spinach treated with chlorinated water (14.40 g kg^{-1}).

3.6. Soluble solids content (SSC)

The effect of treatments, storage time, and their interactions on SSC during cold storage and shelf life were statistically significant. Changes in SSC during storage are presented in Tables 1 and 2. Soluble solids content, which represents soluble sugars in horticultural crop, fluctuated throughout cold storage but decreased in all ozone-treated spinach samples on the last sampling day compared to the initial value (7.4%).On the other hand, the SSC of the control sample increased proportionally as storage time increased because of higher water loss. A similar trend was also observed during shelf life studies in room condition. The average SSC of minimally processed spinach was lower in cold storage when compared to ambient condition. The higher SSC during shelf life studies can be attributed to the higher water loss from spinach depending on high temperature in ambient condition, as reported in previous studies (Uner, 2018; Koyuncu et al., 2018). In all ozone treatments, the relatively higher SSC was obtained from 0.5 ppm ozone treated spinach in both cold storage (7.2%) and room conditions (8.8%). This can be explained by the slowing down of respiration rate (Tables 1 and 2) and senescence activities caused by low (0.5 ppm) ozone dose. Similarly, Fan et al. (2019) reported that the decrease in SSC was due to the senescence of crops during storage. On the other hand, suppressing the respiration rate of horticultural crops delays the conversion of complex carbohydrates to simple sugars, which results in high SSC (Rohani et al., 1997).

3.7. Sensory analysis

Storage time had significant effects on the scores of external appearances, but the effect of treatment and the interaction between these 2 factors were not significant. The external appearance quality of the spinach declined during storage as expected (Tables 1 and 2). Similar results were also reported by Beltran et al. (2005), Sakaldas et al. (2010), and Koyuncu et al. (2018) in leafy vegetables during storage. Spinach with marketable quality (score ≥ 5.00) was only obtained from 0.5 ppm ozone treatment on the 20th day of storage. The higher external appearance scores (7.0 and 6.3) were determined in 0. 5 ppm ozone treated samples during both in cold storage and room condition (Tables 1 and 2). This affirmative effect of low dose ozone can be explained by its suppressing effect on the metabolic activity of spinach compared to other doses and control. As can be seen from Tables 1 and 2, low dose decreased respiration rate and weight loss, which effects the external quality of leafy vegetables. The positive effect of ozone on external quality appears to have disappeared in higher doses (1–2 ppm) having high oxidative properties. In agreement with our findings, ozone treatment delayed sensory quality losses in different fruit and vegetables compared to control samples during storage (Beltran et al., 2005; Çakir et. al., 2014; Uner et al., 2018; Bolel et al., 2019b). In the present study, shelf life studies in ambient condition showed a decrease in external appearance scores compared to cold storage, and the decrease was more evident after 15+1 days. This can be explained by increasing water loss and respiration rates in minimally processed spinach with increasing temperature in ambient conditions.

4. Conclusion

The weight losses of spinach samples in MAP were delayed by low dose ozone during both cold storage and shelf life. Ozone treatments, especially 0.5 ppm, suppressed the respiration rate of minimally processed spinach by slowing down metabolic processes throughout storage.

Low dose ozone treatment preserved the vivid green color of spinach, which is represented with higher h° and SPAD values, better than other doses and chlorinated water (control). Although no significant differences in the external appearance were observed among treatments, 0.5 ppm gave the highest scores at the end of storage. The 0.5 ppm ozone dose seems to be a limit value for maintaining the quality of minimally processed spinach during storage when compared to higher doses and sodium hypochlorite. Minimally processed spinach treated with 0.5 ppm ozone and packaged in MAP could be stored with good quality at 0 °C and 90 ± 5% RH for 15–20 days. When spinach was transferred to ambient condition (1 day) after cold storage, the storage life was reduced to 10–15 + 1 days due to water loss. Ozonated water can be used as an alternative sanitizer to sodium hypochlorite for minimally processed spinach in the cold chain from harvest to market. However, further study is needed to determine the appropriate ozone dose and application type for spinach during storage.

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