

The combined effect of controlled atmosphere with ozone and prochloraz treatment on storage life and quality of pomegranate cv. Hicaznar*

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Abstract

The combined effects of controlled atmosphere (CA) storage and postharvest ozone treatment on storage life and quality of pomegranate cv. Hicaznar were investigated. Pomegranates were harvested and grouped in three lots. Non-treated first group was used as control. Second group was exposed to ozone (4 ppm) gaseous for 6 h at 5 °C. Last group was chemical treatment, dipped into 0.9% prochloraz solution at 5 °C for 10 seconds. After treatments, fruit were stored in CA (5% O₂ - 10% CO₂) conditions at 6 °C and 90±5% relative humidity for 5 months. Weight loss, total soluble solids content, titratable acidity, respiration rate, fruit skin color, decay incidence, chilling injury and sensory evaluation were determined initially and at one month intervals. Fungicide treatment significantly reduced the weight loss and respiration rate of pomegranates. More decay developed on control fruit than those of ozone and fungicide combinations at the end of storage. Ozone and fungicide treatments gave better results in terms of sensory evaluations compared to control group. No chilling injury incidence was recorded in pomegranate during 5 months. As a result, ozone and prochloraz treatments were effective to prolong the storage life and maintain the postharvest quality of Hicaznar pomegranate stored in CA conditions.

Key words: Cold storage, *Punica granatum*, quality, fungicide, ozone

Kontrollü atmosfer ile kombineli olarak kullanılan ozon ve prochloraz uygulamalarının Hicaznar nar çeşidinin depolanma süre ve kalitesi üzerine etkisi

Öz

Çalışmada derim sonrası kombineli olarak uygulanan ozon uygulaması ile kontrollü atmosferde (KA) depolamanın Hicaznar nar çeşidininin depolama süresi ve kalitesi üzerine etkileri araştırılmıştır. Derimi yapılan narlar 3 gruba ayrılmıştır. Uygulama yapılmayan birinci grup kontrol grubu olarak kullanılmıştır. İkinci gruba 5 °C'de 6 s süre ile 4 ppm ozon uygulaması yapılmıştır. Son grup kimyasal uygulama olup, 5 °C sıcaklığındaki %0.9'luk prochloraz çözeltisine 10 saniye süreyle daldırılmıştır. Bütün uygulamalardan sonra meyveler 6 °C sıcaklık ve % 90±5 oransal neme sahip KA (%5 O₂ - %10 CO₂) koşullarında 5 ay süre ile depolanmıştır. Depolama başlangıcında ve birer ay ara ile ağırlık kaybı, suda çözünür kuru madde miktarı, titre edilebilir asitlik miktarı, solunum hızı, meyve kabuk rengi, çürüme oranı, üşüme zararı ve duyusal kalite analizleri yapılmıştır. Fungisit uygulaması depolama boyunca narların ağırlık kaybını ve solunum hızını önemli ölçüde azaltmıştır. Depolama sonunda kontrol uygulamasında diğer iki uygulamaya oranla daha fazla çürüme gözlemlenmiştir. Ozon ve kimyasal uygulamaları duyusal özellikler bakımından kontrol grubuna kıyasla daha iyi sonuçlar vermiştir. Narlarda 5 aylık depolama boyunca üşüme zararı

belirtisine rastlanmamıştır. Sonuç olarak derim sonrası ozon ve prokloraz uygulamaları, KA koşullarında Hicaznar nar çeşidinin derim sonrası kalitesinin korunması açısından etkili olmuştur.

Anahtar kelimeler: Soğukta muhafaza, *Punica granatum*, kalite, fungusit, ozon

Introduction

Turkey is one of the major pomegranate producing countries in the world (Bolel, 2017). Pomegranate, grown in temperate climates, is one of the most important fruit species of many tropical and subtropical countries in the world. It is a non-climacteric fruit and a considerably perishable throughout the period from harvest to consumer table. Postharvest losses of pomegranate, which affect external and internal quality, are still considered remarkable since the weight loss, skin color's discoloration, decay rate and physiological disorders of fruit are high during cold storage (Mirdehghan et al., 2007; Barman et al., 2011; Koyuncu et al., 2019). Pomegranate, with abundant levels of vitamin C, antioxidant and polyphenol compounds, is considered as functional food (Varasteh et al., 2012).

The main fungi results in severe decay in pomegranates after harvest are *Botrytis cinerea*, *Alternaria spp*, *Aspergillus niger*, *Colletotrichum gloeosporioides* and *Cornelia spp* (Palou et al., 2013; Kahramanoglu, et al., 2014). The latent spores placed in the fruit calyx during growing season, in general, become visible after harvest and cause high rate losses in cold storage. Nowadays, it is very difficult to long term cold storage of pomegranate without using fungicide applications. *Botrytis cinerea*, commonly, is controlled by using different postharvest fungicide, but they have a serious threat against human health and the environment even at very low concentrations (Soumya and Bindu, 2012). Naturally, in developed and developing countries, serious objections have occurred against chemical applications. In recent years, natural treatments and storage techniques (such as ozone, edible coating, modified atmosphere packaging, controlled and dynamic controlled atmosphere storage) have been considered alternative solutions for pomegranate storage. Ozone, which has been used as a disinfectant in more than 30 different industry branches in the world for almost 200 years, can be an alternative technique for the cold storage of pomegranate (Graham, 1997). Ozone is an inorganic molecule with

the chemical formula O₃. It has been evaluated for postharvest disease control and other storage uses for many years; in 2001 it was listed as a secondary direct food additive permitted in food for human consumption (Tzortzakakis et al., 2007). Ozone is also known to increase systemic resistance of fruit by controlling decay and increasing antioxidant compounds in grapes (Artes-Hernandez et al., 2003; 2007). The CA rooms are used to retard quality losses such as weight loss, decay rate, firmness etc. and to extend postharvest life of fruit. The effects of ozone gas in combination with CA storage have not been evaluated in pomegranate cv. Hicaznar for maintain fruit quality.

This research aimed to determine the combined effects of CA with ozone and fungicide treatment on the storage life and quality of pomegranate cv. Hicaznar.

Material and Methods

Pomegranates cv. Hicaznar grown in Fethiye (Muğla) was used as fruit material in this research carried out in 2016. Pomegranates were harvested at commercial harvest stage considering to total soluble solids (TSS) content, titratable acidity (TA), fruit size and shape (Karaçalı, 2010). Fruit were transferred immediately to laboratory and then pre-cooled by using cold air. After pre-cooling fruit were divided into three groups. Non-treated first group was used as control group and, second group was exposed to ozone (4 ppm) by ozone generator in a gas-tight cabinet for 6 h at 5 °C. Last group was chemical treatment, dipped into 0.9 % Prochloraz (N-propyl-N-[2-(2,4,6-trichlorophenoxy)ethyl] imidazole-1-carboxamide) solution (at 5 °C) for 10 seconds. Fruit, placed on kraft paper, were allowed to dry at 20 °C. After treatments, pomegranates stored at 6 °C temperature and 90±5 % relative humidity for 5 months in CA (5% O₂ - 10% CO₂) room. During cold storage, the following physical and chemical analyses were performed at one-month intervals.

Chemical and physical analysis

Weight loss: Weight loss of pomegranate was measured based on the initial weight and calculated as percent (%).

Total soluble solids content and titratable acidity: The TSS of pomegranate juice was determined with a refractometer (Digital-Atago Pocket PAL-1) and expressed as %. The TA of pomegranate was measured using 10 mL of juice, which was titrated

(pH meter and titrimeter) with NaOH solution (0.1 mol L⁻¹) to an end-point pH of 8. The acidity of pomegranate was expressed as g 100 mL⁻¹.

Fruit skin color: Fruit skin color was evaluated on the surface of pomegranate with a colorimeter (Minolta Cr 300, 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), a* (represents the degree of red-green color; + a*: red, - a*: green) and b* (represents the degree of yellow-blue color; + b*: yellow, - b*: blue) system, and chroma (C*) and hue angle (h°) values were calculated the following formula: $h^\circ = \tan^{-1} (b^*/ a^*)$, $C^* = [(a^*)^2 + (b^*)^2]^{1/2}$ (Koyuncu et al., 2019).

Respiration rate: Respiration rate was determined using a sample of 500-750 g. Fruit samples were weighed in 5 L airtight jars at room condition (20 °C). After 1-2 h the gas sample was taken from the jars using a gastight syringe and injected into loop of gas chromatographs (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., U.S.A). Respiration rate were measured by Agilent model (6890N) GC using thermal conductivity detector (TCD). The carrier gas flow was 1.7 mL min⁻¹. The temperature of oven was chosen as 40 °C (isothermal). The temperature of the TCD was 250 °C. Results were calculated as mL CO₂ kg⁻¹ h⁻¹ (Koyuncu et al., 2019).

Sensory analysis: The sensory evaluation panel consisted of 7 members of the research staff (Horticulture Department) who were experienced in sensory analysis of fruit. Pomegranate (coded with three-digit numbers) were served at room temperature and analyzed under fluorescent light in a sensory evaluation room. External appearance and flavor (contribute to the overall quality evaluation) were used key sensorial characteristics by panelists. The hedonic scale was used for external appearance and flavor.

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

Flavor (scale 1-5): very poor:1; poor: 2; mild: 3; good: 4; excellent: 5.

Decay and physiological disorders: The fruit taken from storage at one month-intervals were visually checked, and the number of decayed ones was

recorded. The number of decay fruit at the end of the each storage period was calculated as % according to following formula:

$$\text{Decay rate (\%)} = (\text{Decayed fruit}) / (\text{Total number of fruit}) \times 100$$

Physiological disorders were determined according to Selçuk (2012).

Statistical analysis: The completely randomized design (with three replications) was chosen for this experiment. Using software package (SPSS, v.16.0), the general linear model (GLM) was used for statistical analyses. The differences among means (at a significance level of 0.05) were analyzed using Tukey test.

Result and Discussions

Weight loss

Weight loss of fruit is a very important commercial parameter for storage as it directly refers to the decrease in product weight. Water loss from the skin of pomegranate, which has high porosity, affects visual quality of fruit (Selçuk and Erkan 2013). Weight loss of all treated pomegranate increased significantly in parallel with increasing storage period (Figure 1). The lowest weight loss was obtained from CA + ozone (5.55%) combination, which was followed by CA + prochloraz (5.98%) and CA + control (7.17%) at the end of the storage. It is thought that the permeability of skin may be limited by high dose ozone treatment because of its strong oxidant characteristic. Nowadays ozone is practically used in many sectors due to its high oxidant capacity, and because it is much more effective than chlorine and some other antimicrobial agents (Xu, 1999; Horvitz and Cantalejo, 2014). In the present study, weight loss of fruit reached to critical level of 5% at the 4th month of storage (Figure 1). Similarly, Nerya et al. (2006) found more than 5% weight loss in pomegranates stored in controlled atmosphere room for 5 months. However, in general, when the long term storage for pomegranate is taken into consideration, the results of weight loss in our study are consistent with the literature findings (Kirpi, 2010; Selçuk, 2012; Öz et al., 2015).

Respiration rate

It is known that low temperature and CA conditions (low O₂ and high CO₂) decrease respiration rate of horticultural products during cold storage. At present study, the respiration rate of pomegranates was suppressed by the combinations of both CA+ozone and CA+prochloraz compared to

CA+control. The initial high respiration rate ($26.23 \text{ mL CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$) decreased rapidly, especially ozone and prochloraz treated fruit, in the first month of storage and remained at the same levels in the rest of the storage (Figure 1). The positive effects of ozone and prochloraz treatments on respiration rate can be attributed the antimicrobial effects of them. Ozone is also known to increase systemic resistance, which can reduce respiration process in cell, of fruit by increasing antioxidant compounds in fruit (Artes-Hernandez et al., 2003, 2007). In accordance with our results, Erkan and Pekmezci (1997) reported that storage conditions such as low temperature, low O_2 and high CO_2 are effective in suppressing respiration rate in pomegranates.

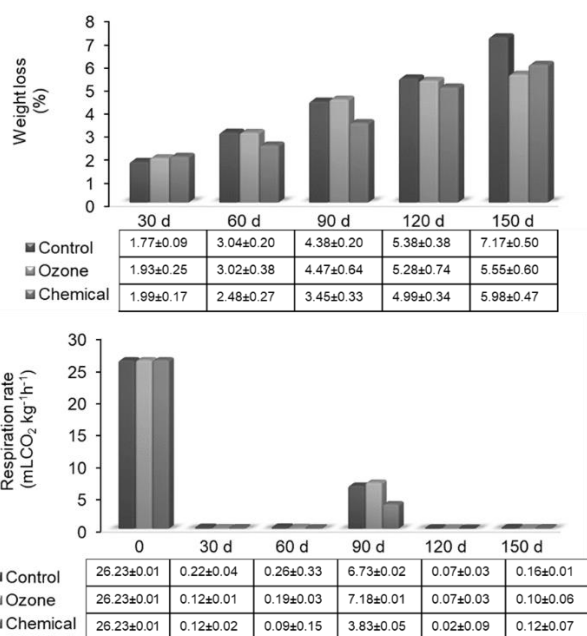


Figure 1. Weight loss and respiration rate of pomegranate cv. Hicaznar during controlled atmosphere storage. Values are mean \pm standard error of triplicate determinations ($n=3$).

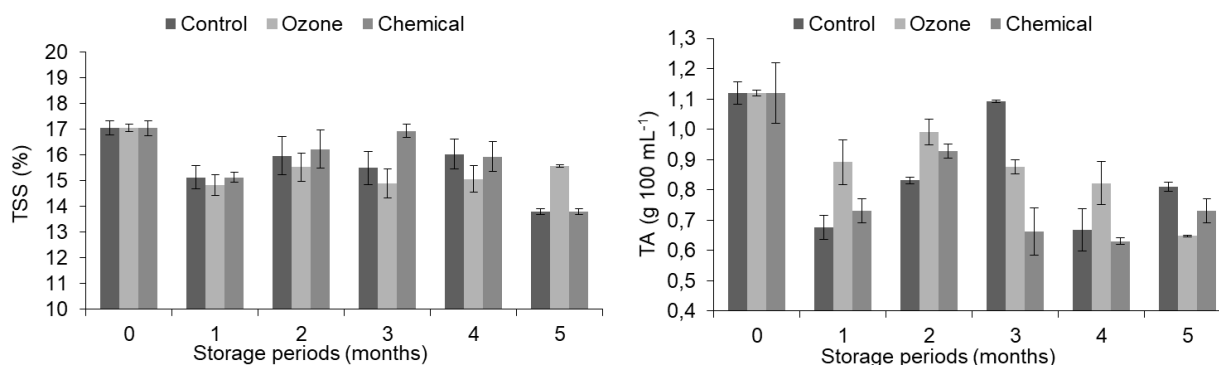


Figure 2. Soluble solids content (TSS) and titratable acidity (TA) of pomegranate cv. Hicaznar during controlled atmosphere storage. Vertical bars represented the standard error of the mean ($n=3$).

Total soluble solids content

The effect of the treatments on the TSS content was not significant, while the effect of storage time was found to be significant (Table 1). The TSS contents of all treated pomegranates slightly fluctuated during storage and decreased compared to initial value at the end of storage. The decrease in the TSS values of fruit treated with ozone was relatively higher (15.4%) than prochloraz (15.8%) and control (15.6%) application (Figure 2). As expected, the decrease in TSS content of the Hicaznar pomegranate during the storage is thought to be due to the use of sugars in respiration process. Similarly, Elyatem and Kader (1984) reported that the TSS contents of pomegranates decreased in parallel with the storage period due to respiration process in fruit. On the other hand, it is known that, the weight loss of pomegranates during storage is not directly from the pomegranate arils, but from the fruit skin. Hence, the TSS increase as a percentage cannot be attributed to weight loss of fruit (Nanda et al., 2001).

Titrateable acidity

The effects of both storage period and treatments on these decreases were significant (Table 1). The highest ($0.92 \text{ g } 100 \text{ mL}^{-1}$) and the lowest ($0.81 \text{ g } 100 \text{ mL}^{-1}$) acidity were obtained from fruit treated with ozone and prochloraz, respectively, according to average values (Figure 2). Similar decreases in acidity of pomegranate throughout the cold storage period were reported by Waskar et al., (1999), Artes et al., (2000), Şen and Eroğul, (2012) and Oğuz et al., (2014). It is desirable to keep TA values of pomegranates close to the initial values during cold storage (Selçuk, 2012). According to this point of view, the combination of CA and ozone treatment gave promising results for TA.

Fruit skin color

The changes in L^* , C^* and h° values of skin color during storage are given in Figure 3. The effect of the treatments on the change of L^* value, which represents the brightness, was significant (Table 1). There were not much variations, except for control, in the average L^* values of pomegranates at the end of the storage as compared with initial values. The L^* value of control group decreased from 57.60 to 53.70, indicating a loss of brightness associated with browning. The best treatment was prochloraz followed by ozone to maintain skin brightness of fruit during storage. It is thought that the oxidant property of ozone was restricted by low oxygen in CA (5%). Similarly, Artes et al. (1996) found that the L^* values of pomegranate fruit remained same levels or increased slightly in controlled atmosphere room.

The effects of both treatments and storage time on C^* and h° values were significant (Table 1). The C^* values, in general, decreased with increasing storage

period, while h° values increased and reached the values changing between 37.30° (prochloraz) and 49.00° (ozone) at the end of 150 days. The highest average C^* value, represents vivid color, was obtained from prochloraz treatment throughout the storage time. This can be explained by that the pomegranates treated with prochloraz lost less weight (Figure 3) and maintained, relatively, quality compared to other applications. It was reported that pomegranates lost their vivid skin color, depending on water loss from skin, after long term cold storage (Selçuk, 2012; Selçuk and Erkan, 2013). As can be seen from Figure 3, the h° values of skin color were higher in ozone treated fruit than those of other treatments. This might be due to the oxidant effect of ozone, which discolored the skin red color of pomegranates. In accordance with present results, Bayram (2007) and Kirpi (2010) indicated increases in h° values of pomegranates during cold storage.

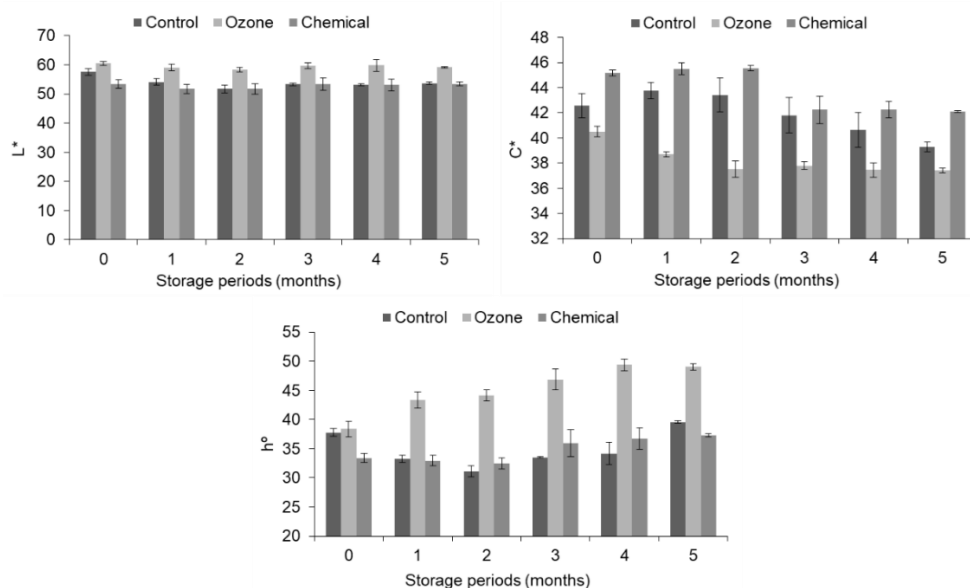


Figure 3. Fruit skin color L^* , chroma (C^*) and hue angle (h°) value of pomegranate cv. Hicaznar during controlled atmosphere storage. Vertical bars represented the standard error of the mean ($n=3$).

Sensory analysis

Significant differences were observed in taste and external appearance of pomegranates throughout storage (Table 1). Panelists gave high scores for external appearance (above 7, except for ozone) and taste (above 4) until the third month of cold storage, and then sensory quality scores decreased depending on treatments in the rest of storage. The higher average scores for both external appearance

and taste were obtained from prochloraz treatment, which maintains quality of pomegranates by controlling many fungi. The taste and external appearance scores of pomegranates decreased with storage time in all treatments, however ozone and prochloraz treated pomegranates maintained marketable scores (above 5 and 3) after 150 days cold storage. Control groups had the lowest sensory quality scores (6.25 and 2.50) at the end of storage

(Figure 4). Similarly, Kirpi, (2010) indicated that control pomegranates (cv. Hicaznar) gave the lowest quality scores in all treatments during cold storage. The Wonderful pomegranates could be stored for 4 months in CA room after fungicide treatments (Nerya et al., 2006). In this trial, ozone, which is applied as an alternative to chemical, was promising treatment compared to control. It can be used for the storage of pomegranates, especially organic ones, but not long term storage.

Decay and physiological disorders

The effects of both storage period and treatments on decay rates were significant (Table 1). It was not determined any decay incidence on pomegranates

until fourth month of storage, but decay rates in ozone treated and control fruit were 13.33% and 7.62%, respectively, after 5 months cold storage. Prochloraz treatment was very remarkable with zero decay incidence throughout whole storage period. Nerya et al. (2006) found that pomegranates stored in CA room maintained their quality better than air storage. On the other hand, it is known that long term (5-6 months) storage of pomegranate in good quality without fungicide is very difficult.

The physiological disorder in pomegranates such as chilling injury and scald were not recorded during storage.

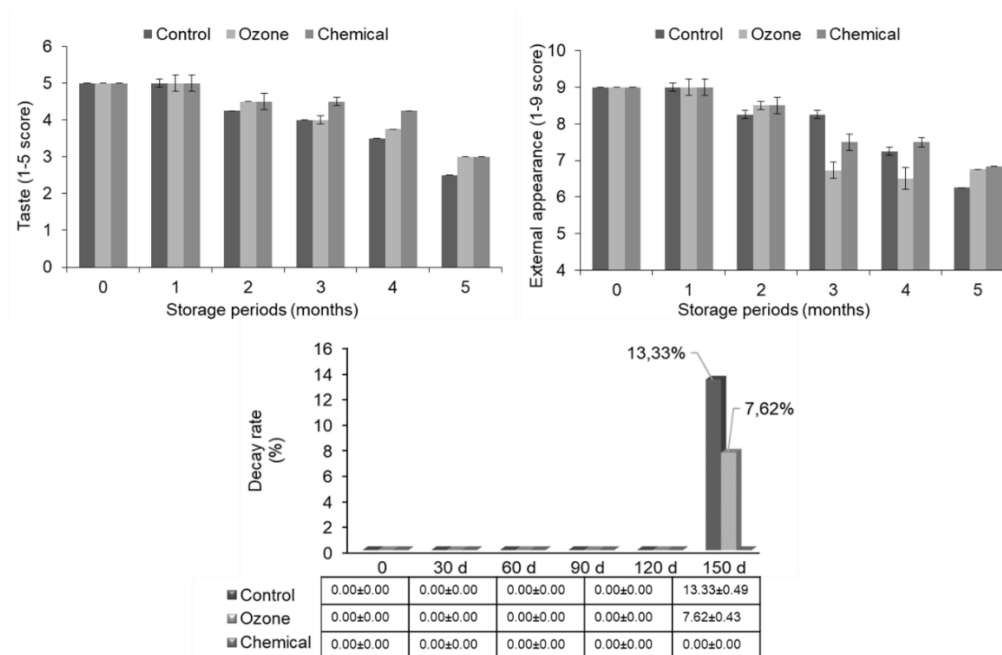


Figure 4. External appearance, taste and decay rate of pomegranate cv. Hicaznar during controlled atmosphere storage. Vertical bars represented the standard error of the mean (n=3). Values are mean \pm standard error of triplicate determinations (n=3). External appearance scale, 1-9 ($\leq 1-4$: poor, ≥ 5 : marketable, 7-8: good, 9: excellent); Taste scale, 1-5 (1: very poor, 2: poor, 3: mild, 4: good, 5: excellent).

Table 1. Anova for dependent variables for treatments, storage period and their interactions

Parameters	Storage period (SP)	Treatments (T)	SP \times T
Weight loss (%)	**	ns	ns
Respiration rate (mLCO ₂ kg ⁻¹ h ⁻¹)	**	ns	ns
TSS (%)	**	ns	ns
TA (g 100mL ⁻¹)	**	*	**
L*	ns	**	ns
Chroma	**	**	ns
Hue angle	**	**	*
External appearance (1-9 score)	**	*	**
Taste (1-5 score)	**	**	ns
Decay rate (%)	**	**	**

Ns represents non-significance at $p < 0.05$; ** represents significant at the 0.01 level; * represents significant at the 0.05 level. TSS: Total soluble solids, TA: Titratable acidity

Conclusions

Ozone and prochloraz treatments gave promising results for sensory evaluations compared to control group during CA storage of pomegranate. Prochloraz treatment had very remarkable results with zero decay incidence during whole storage period. According the decay rate and sensory evaluation, prochloraz and ozone treated fruit, and control group can be stored for 5, 4-5 and 4 months with good quality in CA, respectively. As a result, the combination of CA with ozone gaseous or prochloraz was effective to extend the storability of Hicaznar pomegranate compared to control group. As a non-phytotoxic and effective gaseous, ozone could be a promising tool to extend postharvest life of pomegranate.

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