



Effects of Postharvest Gaseous Ozone Treatment on Quality Attributes and Extending Storage Life of Fresh Cut 'Hass' Avocado Fruits

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Abstract

Ozone is a powerful oxidizing agent, which can kill microorganisms when exposed them to a high enough dosage for a sufficient period. So, this study was carried out during 2020 and 2021 seasons to explore the effect of different doses of gaseous ozone (10, 20, and 40 ppm for 15, 30, and 45 min, respectively) on physicochemical properties, maintaining quality and prolonging storage period of fresh cut "Hass" avocados during cold storage conditions at $5 \pm 1^\circ\text{C}$ with 85-90% RH for 28 days. Fruit measured quality attributes of slices "Hass" avocados were soluble solids content percentage (SSC %), total acidity percentage (TA %), ascorbic acid content (vitamin C, mg/100gFW), chlorophyll a, b and total carotenoids content (TCC, mg/g FW), total phenolic content (TPC, mg /100gFW), and polyphenoloxidase activity (PPO, U/gFW). In comparison to the non-ozonized avocado slices (control), ozone treatments showed a positive effect on extending the storage time of avocado slices with good quality attributes. At the end of storage period, the fruit slices exposed to ozone at 40 ppm for 45 min increased SSC %, PPO and ascorbic acid content, as well as reduced total acidity % and TPC. However, avocado slices exposed to 20 ppm of ozone for 30 min had greater chlorophylls a, b, and total carotenoids. Ozone application could be an effective strategy for keeping up postharvest quality of fresh cut "Hass" avocados and extending the storage life. Therefore, it can be recommended with exposing fresh avocado slices to ozone at 40 ppm for 45 minutes in order to maintain their quality for 28 days under cold storage conditions at $5 \pm 1^\circ\text{C}$ and 85-90% relative humidity.

Keywords: Avocados, Hass, Fresh cut, Gaseous ozone, Fruits quality, Cold storage.

1. Introduction

Avocado (*Persea americana* Mill.) is a popular subtropical climacteric fruit characterized with a green peel and a creamy, buttery pulp, with a high market value, but with a relatively short storage life [1, 2]. Avocados play an imperative part in human nutrition due to its nutritional properties such as oleic, palmitic, linoleic, palmitoleic acids, trace amount of stearic acid, vitamin A, B, C, E, K, and high fiber content [3,4]. Hass avocado cultivar can be highlighted as the foremost imperative one in terms of cultivated region, geographical distribution and consumption [5]. Among the distinctive cultivars of avocados that commercially developed

around the world, 'Hass' proceeds to be the foremost overwhelming due to its nutty flavor and utilitarian properties. Most of researches on postharvest decay and disorders affecting avocado fruit quality during storage and marketing are dedicated to Hass avocado due to its great nutritional value and consumer demand [6]. Postharvest avocado fruit quality deterioration and uneven fruit ripening under cold storage conditions and shelf life are the major challenges that require special attention by fruit industries. Different postharvest physical, chemical and gaseous treatments may be applied to preserve fresh like

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quality with high nutritional value and meet safety measures of fresh produce. These postharvest treatments are typically combined with suitable management of storage temperatures. There are many pre- and post-harvest techniques that have been used to control decay of the fruit [7].

Fruits prepared in "fresh cut" are defined as those that keep up their fresh state, in spite of having suffered physical alterations, undergoing a selection process, washing, peeling and cutting until they approach a 100% usable product that is packaged for presentation to consumers to provide freshness, convenience and quality nutritional status [8]. The quality of the fresh cut products is related to protecting their sensorial characteristics and controlling the microorganisms contaminate, with keeping up of quality properties [9] that include appearance, aroma, flavor and texture [10].

Recently, the global demand has increased to reduce the using the chemical treatments on fruit due to the residue levels which results in serious health concerns. Furthermore, producing natural plants and gases are gaining significant interest to consumers as alternative postharvest treatments of the fruits. Ozone (O₃) aqueous and gaseous applications are other methods that have been researched to preserve fruit fresh cut because it is a strong oxidant and strong antimicrobial agent and has wide application in food industry. The high reactivity, penetrability and spontaneous decomposition to a nontoxic product (O₂) make ozone an alternative that enables food safety from a microbiological side, mainly because ozone has been classified as a GRAS (Generally Recognized As Safe) material in United States and approved by the FDA (Food and Drug Administration) for direct contact with foods. The susceptibility of microorganisms to ozone treatments depends on many factors such as the culture physiological state, pH, temperature, and humidity, additives presence, ozone concentrations and contact times [11,12].

Restaino *et al.* [13] reported that ozone was an extremely effective antimicrobial agent against bacterial pathogens (gram negative and positive), spoilage microorganisms, and yeasts. Ozone reduces the accumulation of oxygen around the fruit, which reduces the respiration rate and leads to biochemical changes that improve the quality of the fruit. It also does not leave residues on the treated fruit and its bactericidal effect depends on its oxidizing effect on the cell membranes of microorganisms [14].

Ozone can be used in air or water as a pretreatment for cold storage or it can be added continuously or intermittently to the storage room atmosphere during storage period for preventing fruit decay

[12]. Several researches have shown that treatment with ozone appears to have a beneficial effect on extending the store life of stone fruits fresh non-cut commodities such as broccoli, apple, grapes, oranges, pears, raspberries and strawberries by reducing microbial populations and oxidation of ethylene [9]. Thus, the objective of this study was to examine the impact of gaseous ozone with different doses on physicochemical properties, keeping up quality and extending the storage life of fresh cut "Hass" avocados in cold storage conditions at $5 \pm 1^\circ\text{C}$ with 85-90% RH.

2. Experimental

2.1. Fruits harvest

Postharvest gaseous ozone treatment was conducted on avocado (*Persea americana* Mill.) fruits cv. Hass obtained from private orchard (Salmiya) at Nubaria, region, Al-Behera governorate, Egypt. Avocado fruits were harvested at maturity stage during 2020 and 2021 seasons from 15 years old trees grown in sandy soil that were similar in growth vigor and subjected to the common horticultural treatments. Mature fruits were harvested to be similar in color, size and free of any noticeable pathological or mechanical injuries. Avocado fruits cv. Hass reached maturity stage when their weight was about 122 - 128 g, dry matter 18.8%, fruit firmness 16.9 and oil content 11.9-12.5%. Fruits were immediately packed and transported to the postharvest laboratory, and then all fruits were washed by tap water and air-dried.

2.2. Preparation of avocado whole fruits

Avocado fruits cv. Hass were washed gently using double distilled water, then were soaked in sodium hypochlorite solution (3%) for 2 min to suppress the fungi load. Lately, were washed using sterile water then dried by a sterile filter paper before treating.

2.3. Preparation of avocado fruit slices

A number of the previous fruits were used for fruit slices preparation. The fruits were cutting into five vertical slices, where the seeds were discarded. The slices were washed using a sterile phosphate buffer saline before coating and emerging them in the preservation solution.

2.4. Ozone treatments

Avocado fruits were treated with ozone gas using an ozone generator (Model OZO6 VTTL OZO Max Ltd, Shefford, Quebec Canada). Fruit samples (about 120 - 150 g) were placed in a square

container with a sealed cap, connected to the inlet valve at the initial and outlet valve at the opposite sides. Fruit slices were exposed to ozone gas at 10 ppm /15 min, 20 ppm /30 min, and 40 ppm /45 min except control slices that was left without ozone treatment. Treated fruits were stored in controlled temperature rooms at 5 ± 1 °C and 85-90% relative humidity for 28 days. Each treatment consists of three replicates and each replicate consist of three fruits. The quality of the fruit slices was evaluated every 7 days from the beginning of cold storage (0 time) till the end of the storage period (28 days) in both seasons of the study and the data were presented as average of two seasons.

2.5. Fruit quality assessment

2.5.1. Soluble solids content (SSC, %): SSC % was measured using a T/C hand refractometer Instrone, Brix readings 0 - 30 ranges (Model 10430, Bausch and Lomb Co. Calif., USA) and expressed in percentage.

2.5.2. Titratable acidity (TA, %): Total acidity content (expressed as *oleic acid*) was determined by titrating 5 ml juice against 0.1N sodium hydroxide using phenolphthalein as an indicator.

2.5.3. Ascorbic acid content (Vitamin C, mg/100g FW): Ascorbic acid content was measured by using 2, 6 dichlorophenol indophenols as the method described by A.O.A.C. [15].

2.5.4. Chlorophyll a, chlorophyll b and total carotenoids contents (mg/g FW): Chlorophyll a, b and total carotenoids content in the pulp of avocado fruits were measured spectrophotometrically according to the methods described by Wellburn [16].

2.5.5. Total phenolic content (TPC, mg / 100g FW): The total phenolic content was determined in *methanolic extract of fresh fruit* by using the Folin–Ciocalteu assay as described by Villa-Rodríguez et al. [17]. Total *phenolic content* was calculated as mg pyrogallol per 100 g fresh weight (FW).

2.5.6. Polyphenoloxidase enzyme activity (PPO, Ug/ FW): The activity of polyphenol oxidase was determined as reported by Vargas-Ortiz [18]. For each treatment, 5 g of mesocarp was used to prepare the enzyme extract. Five hundred μ L of 20 mM catechol was added as the substrate with 900 μ L of 50 mM phosphate buffer (pH 6.5), and 100 μ L of the enzyme extract. The mixture was incubated for 20 min at 25 °C, and the reaction was stopped with the addition of 500 μ L of 10% TCA. The absorbance was read at 410 nm using a spectrophotometer Jenway 6715 UV–Vis (USA). The PPO activity was reported as the increase in absorbance after the reaction time for 100 μ L of extract.

There was a gradual and significant decrease in the total acidity percentage of avocado slices cv.

2.6. Statistical analysis

The treatments were arranged as experiment in a randomized complete design. All data were subjected to statistical analysis according to the procedures reported by Snedecor and Cochran [14] and means were compared by Duncan's multiple range tests at the 5 % level of probability according to Duncan [15] in the two seasons of experimentation.

3. Results and discussion

3.1. Soluble solid content (SSC, %)

The soluble solid content percentage of 'Hass' avocado slices was gradually decreased significantly with the advance of the cold storage period at 5 ± 1 °C and 85-90% relative humidity at all gaseous ozone doses and also the non-ozonized slices (control) during the average of two seasons of this study (Fig. 1). Ozone doses treatments have an obvious effect in maintaining SSC% compared to non-ozone-treated avocado slices during 28 days of storage. At the end of the storage period, there was no significant difference between the different doses of ozone in the percentage of soluble solid content.

These results are concur with the findings of Xuan *et al.* [20] who found that the sugar content in avocado flesh tissue was declined sharply during fruit ripening with the start of oil accumulation, especially C₇ polysaccharides which lead to SSC % reduction. Also, they indicated that C₇ polysaccharides play an imperative part, not only in metabolic processes associated with fruit growth, but also respiration processes that related to postharvest physiology and fruit ripening. Our results are in agreement with Yassin *et al.* [21] who found that the decrease of SSC % in avocado pulp with an extended storage period could be attributed to the decomposition of the complex into soluble compounds such as sugars which are the main components of soluble solid content in the fruit. The findings are correspond with Zhang *et al.* [22], who described that ozone increases the soluble solids content in strawberries during cold storage, which may be attributed to the influence of ozone treatment in slowing the respiratory rate and ripening processes. These results are in line with those of Tzortzakis *et al.* [23]; Barakat *et al.* [24]; EL-Hadidy [25] who demonstrated that, soluble solids contents of orange fruits were increased significantly by postharvest treatments with ozone.

3.2. Total acidity percentage (TA, %)

'Hass' as the storage periods progressed at 5 ± 1 °C and 85-90% RH under all ozone treatments (Fig. 2).

Ozone treatment with different doses showed that TA was maintained lower than the control during the cold storage period (28 days) with significant differences at most intervals. After 28 days of storage, avocado slices exposed to high concentration of ozone (40 ppm/45 min) recorded the lowest significantly percentage of total acidity (0.37%) compared with the other doses. El-Oraby and Ekbal [26] reported similar results on Thompson Seedless grapes and Florida Prince Peach, Whangchai *et al.* [27] on Sai Nam Pung tangerine; EL-Hadidy [25] on Washington

Navel orange who reported that the high doses of ozone treatments significantly reduced the acidity of fruit juice. Also, the results are in line with the findings of the previous study of Cayuela *et al.* [28] who indicated that ozone treatments significantly decreased the acidity in grapes 'Superior Seedless' samples that exposed to continuous or intermittent ozone at 2 ppm during storage at 5°C.

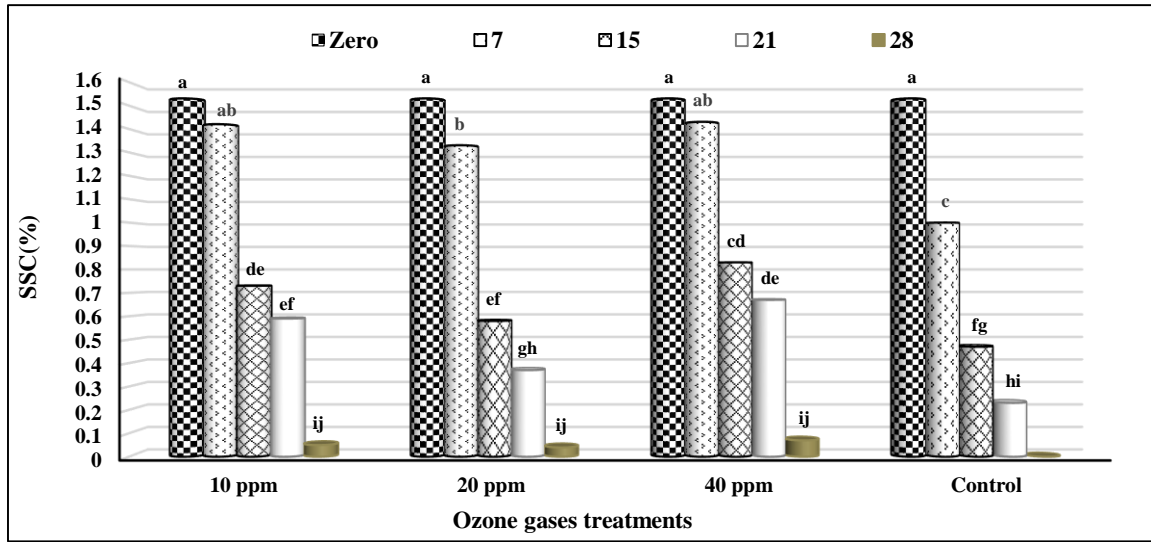


Figure 1: Effect of gaseous ozone doses on soluble solid content (%) of fresh cut 'Hass' avocado fruits stored at 5 ± 1°C and 85-90% RH

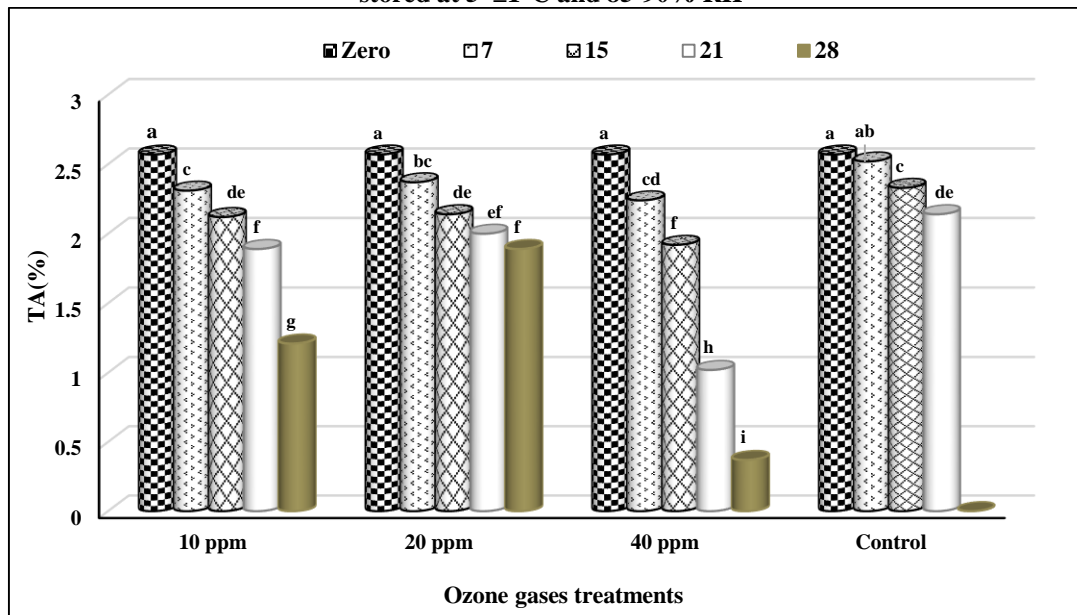


Figure 2: Effect of gaseous ozone doses on total acidity % of fresh cut 'Hass' avocado fruits stored at 5 ± 1°C and 85-90% RH

3.3. Ascorbic acid content (VC, mg/100ml)

Throughout the storage periods, vitamin C content of avocado fresh cut stored at $5 \pm 1^\circ\text{C}$ and 85-90 % RH exhibited an obvious tendency to decline with all treatments including the control (Fig. 3). The statistical analysis indicated that there was a significant difference between the ozone doses in preserving the vitamin C content compared to the non-ozonized slices during the storage days. After 28 days of the storage, slices exposed to ozone 40 ppm / 45 min recorded the highest value of vitamin C (19.43 mg/100 ml), followed by those exposed to ozone at 10 ppm / 15 min (17 mg/100 ml) and followed by ozone at 20 ppm/30 min (13.37 mg/100 ml).

This reduction in ascorbic acid content is possibly due to the oxidative reactions in tissues of slices avocado during storage. A similar behavior was found by Ayón-Reyna *et al.* [29] who suggested that the ascorbic acid content was converted into dehydroascorbic acid through a reversible reaction because the fruits were exposed to air, and possibly later was converted into 2-3-dicetogulonic acid. Ozone treatments were more effective in keeping the ascorbic acid level of avocado slices stable throughout storage. These results are similar to those achieved by Pérez *et al.* [30] on strawberries; Ladaniya [31] on lime; Barboni *et al.* [32] on kiwifruit and Khalil [33] on Zaghoul date palm; EL-Hadidy [25] on Washington Navel orange since ozone gas treatments delayed the degradation of ascorbic acid content and increased its concentration in the fruit at cold storage conditions. Klopotek *et al.* [34]; Allende *et al.* [35]; Alwi and Ali [36] explained the increase in ascorbic acid content of fruits exposed to O_3 to the high oxidative capacity of ozone generate toxic molecular species, that acting as a potent phytotoxic agent, causing activation of the fruit defense system (anti-oxidative system) that promotes the biosynthesis of ascorbic acid from the carbohydrate pool [30, 37] to avoid the oxidative activity caused by ozone.

3.4. Flesh pigments content (chlorophyll a, b and total carotenoids)

Pigments are important factor that contribute to the appearance and health properties of avocado fruits

and the oil extracted from this fruit [38]. When measuring the chlorophyll content a and b in avocado slices exposed to ozone treatment and stored at $5 \pm 1^\circ\text{C}$ and 85-90 % RH for 28 days, it was noticed that both of them were decreased significantly over the storage time (Figs 4 and 5), whereas total carotenoids content was increased significantly with extended the storage period (Fig. 6).

Avocado slices exposed to ozone at 20 ppm/30 min recorded the highest levels of chlorophyll a, b and total carotenoids content (0.457, 0.399 and 2.041 mg/g FW, respectively). This result was in line with Careli-Gondim *et al.* [39] who found a decrease in chlorophyll 'a' and chlorophyll 'b' content and an increment in carotenoids in avocado pulp after 20 days of storage at 10°C . These changes in pigments may be associated with the fruit ripening process [40], where chlorophyll is degraded and the carotenoids are unmasked. Chlorophyll degradation is a key catabolic process for green fruit ripening [41], and chlorophyllase works by removing a phytol group, resulting in chlorophyllidium, the initial degradation product. The central Mg ion is then removed by Mg-dequelatase, and the pheoforbidiol is oxidised by pheophorbide oxygenase. This produces a main fluorescent chlorophyll catabolic product, which is then transformed into non-fluorescent chlorophyll catabolites, allowing the carotenoids to be seen [42]. Our findings indicate that chlorophyll content in the slices of avocado exposed to ozone was higher than in the untreated avocado. This is consistent with Lu *et al.* [43] who demonstrated that O_3 effectively suppresses the activity of the chlorophyll-degrading enzyme, which reduces the degree of chlorophyll hydrolysis that makes the chlorophyll content of ozonized broccoli fresh cut higher than non-ozone treated.

Our findings demonstrated that ozone treatments enhanced the development of carotenoids, this agree with EL-Hadidy and Nagy [44] who noticed that carotene content of apricot fruits was increased gradually with the progress of storage period at 0°C and 90% (RH) under ozone treatments.

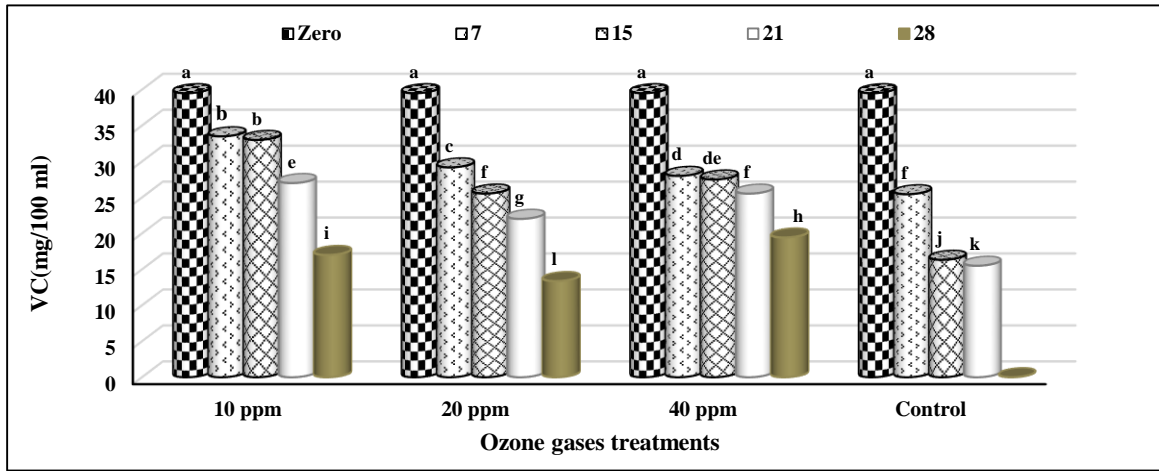


Figure 3: Effect of gaseous ozone doses on vitamin C content of fresh cut 'Hass' avocado fruits stored at $5 \pm 1^\circ\text{C}$ and 85-90% RH

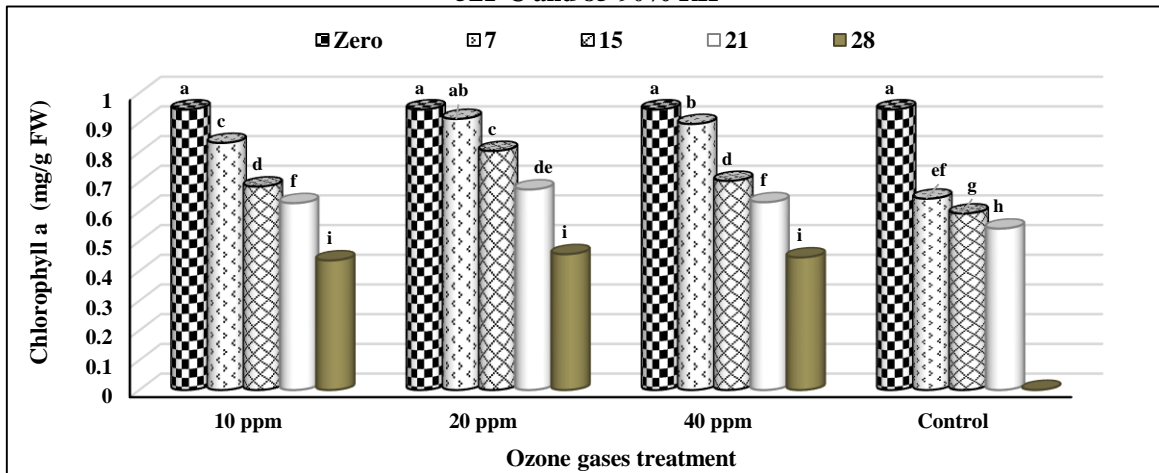


Figure 4: Effect of gaseous ozone doses on chlorophyll a content in fresh cut 'Hass' avocado fruits stored at $5 \pm 1^\circ\text{C}$ and 85-90% RH

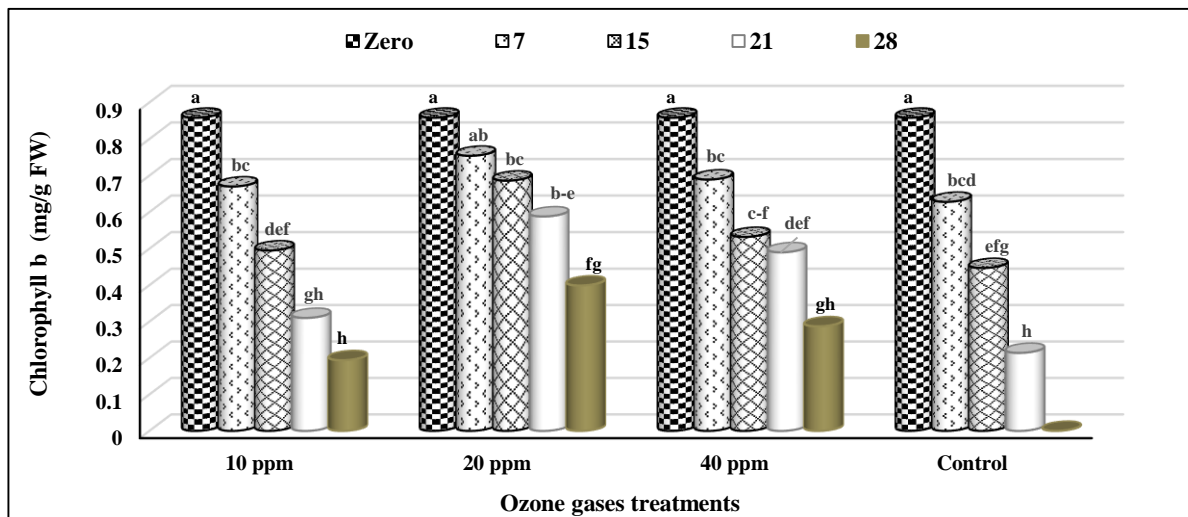


Figure 5: Effect of gaseous ozone doses on chlorophyll b content in fresh cut 'Hass' avocado fruits stored at $5 \pm 1^\circ\text{C}$ and 85-90% RH

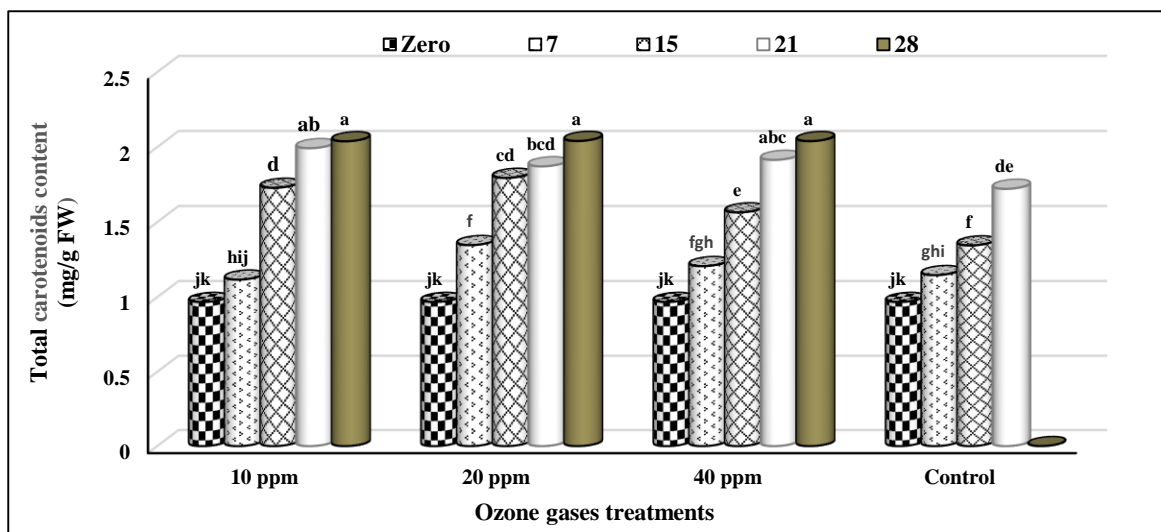


Figure 6: Effect of gaseous ozone doses on total carotenoids content in fresh cut 'Hass' avocado fruits stored at $5 \pm 1^\circ\text{C}$ and 85-90% RH

3.5. Total phenolic content (TPC, mg /100g FW)

The initial phenolic value in fresh cut 'Hass' avocado fruits stored at $5 \pm 1^\circ\text{C}$ and 85-90 %RH was approximately 190.64 mg /100g FW (Fig. 7). With extended the storage period, this value was declined significantly with control and ozone-treated samples, and reached the lowest values at 28 days. At the end of storage period, the avocado slices exposed to ozone at 10 ppm / 15 min showed the highest mean of TPC (52.41 mg /100g FW), followed by those exposed to ozone at 40 ppm/45 min (51.84 mg/100g FW) without significance between them compared to non-ozonized slices (control). The decrease in total phenolic content observed in this study for both control slices and ozone-treated slices could be due to the oxidation by PPO. These results are in agreement with the previous reports by Kumar *et al.* [45]; Ayón-Reyna *et al.* [29] about pineapple, who found that

phenolic compounds uses as substrates by the PPO, produced compounds that provoke the browning in the pineapple slices surface.

These findings are in line with Cayuela *et al.* [28] on grapes; Alothman *et al.* [46] on banana and pineapple fresh cut; Minas *et al.* [47] on kiwi and Khalil [33] on 'Zaghloul' date palm, since all of them concluded that ozone treatment induced higher total phenolic contents. Besides, Ali *et al.* [48] reported that a higher increase in content of total phenolic was detected in response to ozone at 1.5-5 ppm for 96 h in papaya when compared with the control fruits in non-ozonized environment. This enhancement might be attributed to the function of ozone in excess oxidative stress and production of reactive oxygen species (ROS), which then needed to be scavenged by antioxidants, e.g. phenolic compounds.

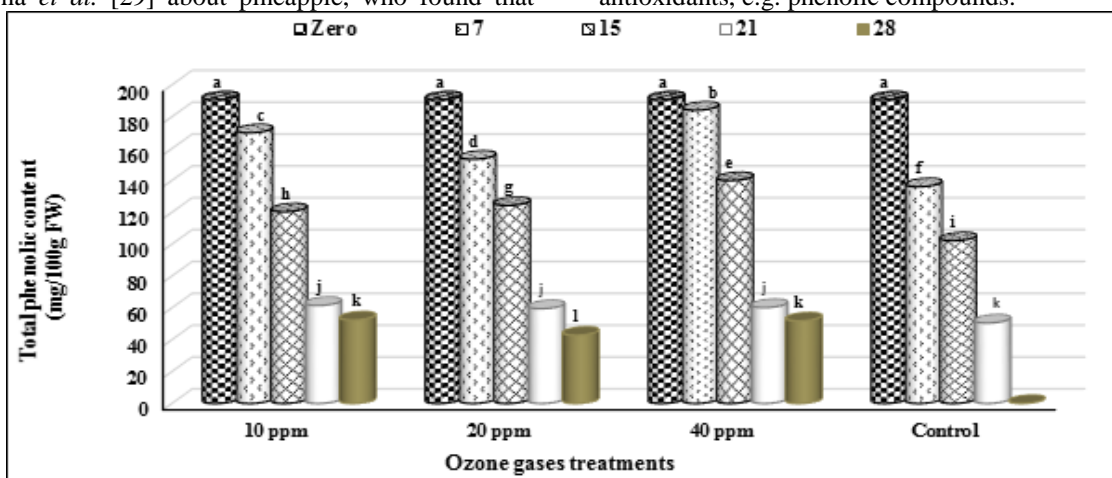


Figure 7: Effect of gaseous ozone doses on total phenolic content of fresh cut 'Hass' avocado fruits stored at $5 \pm 1^\circ\text{C}$ and 85-90% RH

3.6. Polyphenoloxidase activity (PPO, Ug/FW)

The statistical analysis showed a gradual and significant increase in polyphenoloxidase activity (PPO) for avocado slices cv. 'Hass' during cold storage at $5 \pm 1^\circ\text{C}$ and 85-90% RH due to the different doses of ozone till reached the highest values at the end of the storage period (28 days) (Fig. 8). All-doses of ozone had higher PPO activity than the control (non-ozone treated) during the storage days with significant differences between them, but at the end of storage period the differences were not significant in PPO between ozone doses.

These results are in agreement with the findings by Ong *et al.* [49], who reported that the

polyphenoloxidase (PPO) activity were higher in papaya fruits treated with ozone than in untreated fruits throughout the storage period. The greatest changes in enzyme activities were recorded with the highest dose of ozone ($5 \mu\text{L L}^{-1}$). Also, previous results showed that the fresh cut lettuce treated with ozone during storage showed significantly lower ($P < 0.05$) PPO enzymatic activities than the untreated samples [50]. This decrease in PPO activity could be due to the high oxidation potential of ozone. Saftner *et al.* [51] considered ozone to be an efficient organic matter oxidant that could reduce the microbial content, resulting in reducing the rate of respiration and decreasing the enzyme synthesis.

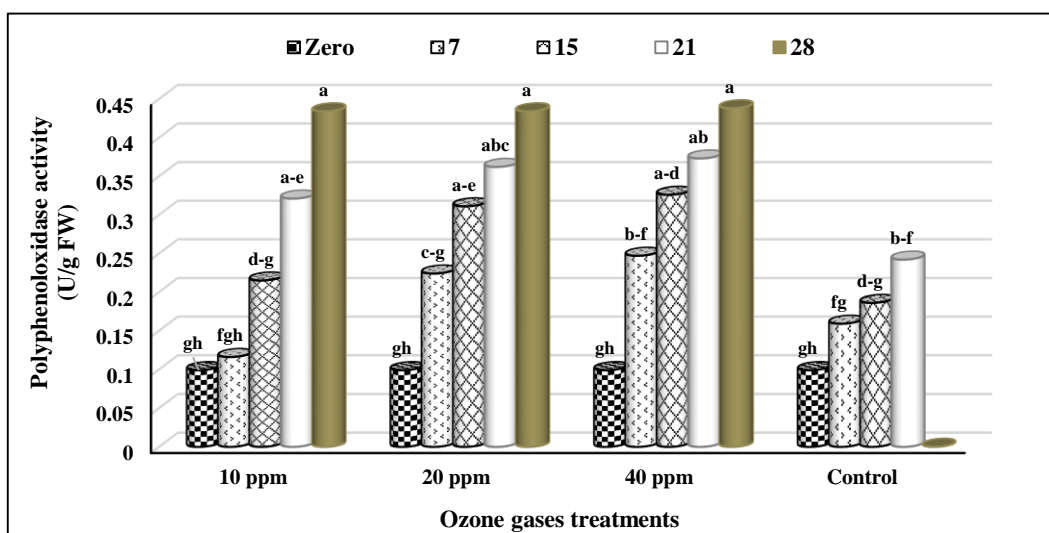


Figure 8: Effect of gaseous ozone doses on PPO activity of fresh cut 'Hass' avocado fruits stored at $5 \pm 1^\circ\text{C}$ and 85-90% RH

4. Conclusions

In general, ozone treatments preserved the quality of fresh cut 'Hass' avocado slices in cold storage as compared to the untreated slices. Soluble solids, PPO enzyme activity, ascorbic acid content and flesh pigments content (chlorophylls a, b and total carotenoids) were higher in the treatments with ozone. A reduction in total acidity percentage and total phenolic contents were produced by exposing to ozone treatments. Based on these results, it can be recommended to apply ozone at 40 ppm for 45 min in order to maintain the quality of fresh cut 'Hass' avocados for 28 days during the cold storage at $5 \pm 1^\circ\text{C}$ and 85-90% RH.

5. Conflicts of interest

The authors declare that they have no conflict of interest.

6. Formatting of funding sources

National Research Centre.

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