



Aqueous ozone controls decay and maintains quality attributes of strawberry (*Fragaria × ananassa* Duch.)

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Abstract Investigations were made on the changes in physical and biochemical attributes, fruit decay and storage life of ‘Winter Dawn’ strawberry fruits in response to aqueous ozone dip treatment for different exposure times. Fruits were subjected to 0.1 ppm aqueous ozone for different time intervals (1–4 min). The treated strawberries were air dried and stored under ambient (25 ± 2 °C and 45–50% RH) and low temperature (2 ± 1 °C and 90% RH) conditions. Results revealed that treatment of strawberry fruits with aqueous ozone @ 0.1 ppm for 2 min resulted in 21% lower weight loss, 16% higher firmness and 15% lesser change in fruit colour during 2 days in ambient storage. Under low temperature storage, 2 min ozone treated fruits exhibited ~ 21% lower PLW, 19% higher firmness and 46% lesser colour change as compared to control fruits during 14 days of storage. Fruit decay reduced significantly under both low and cold storage conditions. Thus, it can be concluded that application of aqueous ozone for 2 min was able to retain the strawberry fruit quality and extend its storage life till 14 days under

low temperature storage and 2 days under ambient storage conditions.

Keywords Strawberry · Ozone · Storage life · Decay · Anthocyanins · Quality

Introduction

Strawberry (*Fragaria × ananassa* Duch.) is a non-climacteric fruit, famous for its attractive appearance, taste and high nutritional value. It is a perishable fruit, susceptible to attack by postharvest pathogens causing significant losses (Hajji et al. 2018). Increasing consumer health concerns has led to the decline in the demand of fungicide treated fruits and adoption of alternative eco-friendly techniques to reduce decay incidence and enhancement of postharvest storage life. Ozonation is one such potential clean technology to maintain fruit safety and quality that leaves no toxic residue within the fruits. With its approval as a Generally Recognized as Safe (GRAS) ingredient by United States Food and Drug Administration (USFDA) in 2001 (Anonymous 2001), application of ozone (O₃) has gained recognition as a sanitizing agent. It has an oxidizing potential 1.5 times greater than that of chlorine and is much effective than chlorine as it can significantly control a wider spectrum of microorganisms without leaving any harmful residue (Zhu 2018). Commodities can be exposed to continuous or intermittent gaseous ozone (García-Martín et al. 2018; Minas et al. 2014) in the storage chambers or can be subjected to dissolved ozone in aqueous phase used for the treatment (Murray et al. 2018; Liu et al. 2016). Ozone inactivates cells as a result of oxidative stress caused by reactive oxygen species (ROS) such as OOH·, OH· and H₂O₂ which are produced by ozone

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decomposition (Tzortzakis and Chrysargyris 2016). Effect on microbial cells is manifested by irregular cell structure (Ong et al. 2013) due to oxidation effects of ozone and induced ROS on lipid and protein molecules embedded in the bacterial cell membrane. The production of lipid peroxides and oxidation of sulfhydryl groups of protein increases membrane fluidity, reduces cell integrity and leads to cell wall rupture, excessive nutrient loss and cell death. Several studies have shown that treatment of fruits with ozonated water can extend the storage life and help to improve the food quality (Concha-Meyer et al. 2015; Pandiselvam et al. 2018; Lv et al. 2019). The present investigation was laid out in an attempt to study the beneficial effects of ozone in aqueous solution on strawberry fruits towards delaying postharvest disease incidence and maintenance of quality during storage.

Materials and methods

Raw material procurement and ozone treatment

Strawberry fruits cv. ‘Winter Dawn’ were harvested at 70% colour development stage in the month of December from a private orchard of village Palla, Delhi. Ozone was generated by corona discharge method using Oz-Air 2.0 instrument. The generated gas was dissolved in water and this aqueous ozone was used to treat the strawberry fruits as a sanitizing agent. The fruits were subjected to 0.1 ppm aqueous ozone for different time intervals (1–4 min). Control fruits were dipped in water. Strawberries were air dried and stored in plastic punnets under ambient temperature (25 ± 2 °C and 45–50% RH) and low temperature (2 ± 1 °C and 90% RH) conditions. The analysis was done daily for fruits stored under ambient conditions and after every 2 days interval for low temperature stored fruits for various physico-chemical attributes. All readings were taken in replicates.

Observations recorded and methodology

Physiological loss in weight

Physiological loss in weight of the strawberry fruits during storage was determined by weighing the fruits at different time intervals and calculated by using the following formula:

$$\text{PLW (\%)} = \frac{\text{Initial weight} - \text{Weight after known storage period}}{\text{Initial weight}} \times 100$$

Fruit decay

Fruit decay was determined visually by counting the diseased and healthy strawberries in each treatment at every interval and expressed as percentage.

Firmness

Fruit firmness was determined by a Stable micro systems (UK) texture analyzer using a puncture probe with pre-test speed of 5 mm/s. Maximum force attained during the puncturing of strawberry fruits was noted to denote the fruit firmness and expressed in Newton (N).

Biochemical attributes

Fruit quality attributes in ‘Winter Dawn’ fruits such as ascorbic acid content and total phenolic content were determined according to standard methods (Ranganna 2007). The total anthocyanin content was determined using the pH-differential method (Wrolstad et al. 2005) using two buffer systems—potassium chloride, pH 1 (0.025 M) and sodium acetate, pH 4.5 (0.4 M). The pigment content in fruits was calculated and expressed as pelargonidin 3-5-diglucoside per kg fresh weight.

Statistical analysis

The experiment was conducted in a factorial completely randomised design with three replications, each replication having 50 strawberry fruits. Data analysis was performed using SAS 9.3 software and Duncan’s Multiple Range Test (DMRT) to compare the treatments along the storage duration.

Results and discussion

Physiological loss in weight (PLW)

A reduction in fruit weight during storage is primarily attributed to the loss of moisture during respiration and transpiration processes (Kumar et al. 2017). As shown in Table 1, all treated as well as untreated (control) ‘Winter Dawn’ strawberry fruits exhibited loss in weight both during ambient and low temperature storage conditions. Interestingly, ozone treated strawberry fruits demonstrated lower weight loss than control fruits. At the end of 2nd day of storage under ambient conditions, ozonated water dipped (2 min) strawberry fruits showed least PLW (5.24%), which was nearly 18% lower than control fruits. Likewise, under low temperature storage conditions also, PLW was the lowest (5.67%) when fruits were exposed to aqueous

Table 1 Influence of aqueous ozone dip treatment on PLW (%) of strawberry fruits cv. ‘Winter Dawn’ during storage

Time of aqueous ozone dip (min)	Ambient (25 ± 2 °C and 45–50% RH)			Low temperature (2 ± 1 °C and 90% RH)							
	Storage period (days)			Storage period (days)							
	0	1	2	0	2	4	6	8	10	12	14
0	0 ^d	3.67 ^{bc}	6.17 ^a	0 ^g	1.68 ^{jih}	2.04 ^{gfi}	2.48 ^{gfi}	2.90 ^{gfi}	3.50 ^{gfdec}	4.81 ^{bac}	6.32 ^a
1	0 ^d	2.97 ^{bc}	5.30 ^{ba}	0 ^g	1.43 ^{ji}	1.58 ^{jih}	1.92 ^{gfi}	2.20 ^{gih}	2.91 ^{gfi}	4.29 ^{bdec}	5.68 ^{ba}
2	0 ^d	2.82 ^c	5.24 ^{ba}	0 ^g	1.37 ^{ji}	1.39 ^{ji}	1.77 ^{gfi}	2.21 ^{gfi}	2.92 ^{gfdeih}	4.25 ^{bdec}	5.67 ^{ba}
3	0 ^d	3.26 ^c	5.51 ^{ba}	0 ^g	1.59 ^{jih}	1.79 ^{gih}	2.15 ^{gih}	2.62 ^{gfi}	3.31 ^{gfdech}	4.66 ^{bdac}	6.18 ^a
4	0 ^d	3.61 ^{bc}	5.84 ^a	0 ^g	1.70 ^{jih}	2.05 ^{gfi}	2.41 ^{gfi}	2.81 ^{gfi}	3.56 ^{fdec}	4.36 ^{bdec}	6.28 ^a

*Means with same superscript are not significantly different at $p \leq 0.05$

ozone for 2 min and highest (6.32%) in control fruits. Exposure of strawberry fruits to ozone for longer duration (3–4 min) resulted in slightly higher weight loss than exposure to lower time period (1–2 min) (Table 1). Although there is no report in the literature to support these results, however, we assume that exposure of strawberries to longer duration to ozone might have resulted in tissue damage which caused increased water loss from the fruits. Salvador et al. (2006) have also stated previously that exposure of fresh persimmon to gaseous ozone may negatively affect physiological traits such as respiration or membrane stability, thus reducing product quality and shelf life. A decline in PLW with a progressive increase in storage period may be attributed to the loss of moisture due to enhanced rates of respiration and transpiration (Kumar et al. 2017). However, differences were not significant at the end of storage both under ambient as well as low temperature conditions. Our results corroborate with the findings of Zhang et al. (2011), who reported that ozone treated strawberries maintained low PLW than untreated ones during cold storage.

Fruit firmness

Strawberry is a very delicate fruit and maintenance of its firmness during storage is an important criterion for retaining its acceptability. It is evident from the data presented in Table 2 that although, under ambient storage strawberry fruits demonstrated a reduction in firmness under all treatment combinations, but firmness was maximum (1.36 N) in fruits treated with aqueous ozone treatment given for 2 min. Ozone exposure for 4 min resulted in 4.4% higher loss in firmness (1.30 N) of strawberry fruits than those exposed for lesser time. Similarly, under low temperature storage conditions also, firmness was the highest (1.20 N) when fruits were exposed to aqueous ozone for 2 min and lowest (1.01 N) in control fruits

(water dip) (Table 2). The major reason for reduction in firmness of strawberry fruits might be due to changes in the structural components of the cell wall, turgor pressure and intercellular adhesion as reported by Fraeye et al. (2009) and Kumar et al. (2018) in strawberry and plum, respectively. Moreover, a gradual cellular degradation during storage due to senescence process may also be a contributing factor for firmness loss (Kumar et al. 2018). Furthermore, ozone is oxidative in nature, which is responsible for inactivating fruit softening enzymes such as pectin methylesterase, which helps in maintaining fruit firmness during storage (Rodoni et al. 2010). Also ozone has been attributed to cause suppression of ethylene gas production that is responsible for fruit softening (Minas et al. 2018), hence helping in better retention of firmness. In our results, we observed that aqueous ozone dip of strawberry for lower duration (2 min) maintained better firmness than longer duration (3–4 min) indicating that ozone (0.1 ppm) exposure for less than 2 min duration was favourable in maintaining fruit firmness. Longer ozone exposure might have degraded the cuticular components at a faster rate than by the exposure for lesser time as reported by Zhang et al. (2011) in strawberry.

Fruit decay

Strawberry fruits are highly perishable and prone to microbial decay leading to huge postharvest losses. In our study, ozone treatment significantly reduced decay in ‘Winter Dawn’ strawberry fruits. Decay was lowest (22.2%) for strawberry fruits treated with ozone for 2 min and highest (44.4%) for untreated strawberry fruits under ambient storage (25 ± 2 °C) of 2 days (Fig. 1). Similarly, the beneficial effect of aqueous ozone in retarding decay of strawberry fruits was evidenced under low temperature storage also. Interestingly, under low temperature storage, no visible decay was observed up to the end of 1st week in

Table 2 Impact of aqueous ozone on firmness (N) of strawberry fruits cv. ‘Winter Dawn’ during storage

Time of aqueous ozone dip (min)	Ambient (25 ± 2 °C and 45–50% RH)			Low temperature (2 ± 1 °C and 90% RH)							
	Storage period (days)			Storage period (days)							
	0	1	2	0	2	4	6	8	10	12	14
0	1.41 ^{ba}	1.31 ^{edf}	1.26 ^f	1.40 ^{bdac}	1.37 ^{ebdagcf}	1.33 ^{ejdihgcf}	1.30 ^{mjlhngkn}	1.26 ^{mjlqpkn}	1.22 ^{rqposn}	1.17 ^{rqs}	1.01 ^{ba}
1	1.42 ^a	1.39 ^{bac}	1.34 ^{edc}	1.38 ^{ebdacf}	1.35 ^{ebdhgcf}	1.33 ^{ejdihgkf}	1.30 ^{mjlhngkn}	1.27 ^{mjlipkn}	1.24 ^{mrlqpon}	1.21 ^{rqpos}	1.18 ^{bac}
2	1.40 ^{bac}	1.37 ^{bdac}	1.36 ^{ebdac}	1.39 ^{ebdac}	1.37 ^{ebdagcf}	1.35 ^{ebdhgcf}	1.32 ^{ejdihgkf}	1.30 ^{mjlhngkn}	1.27 ^{mjlipkn}	1.24 ^{mrlqpon}	1.20 ^a
3	1.39 ^{bac}	1.35 ^{ebdc}	1.31 ^{edf}	1.37 ^{ebdagcf}	1.34 ^{ebdhgcf}	1.31 ^{mjlhngkf}	1.28 ^{mjlhngkn}	1.25 ^{mrlqpkn}	1.21 ^{rqpos}	1.17 ^{rs}	1.15 ^{bac}
4	1.40 ^{bac}	1.35 ^{ebdc}	1.30 ^{ef}	1.35 ^{ebdhgcf}	1.31 ^{ejlhngkf}	1.27 ^{mjlipkn}	1.23 ^{mrlqpon}	1.19 ^{rqps}	1.15 ^{ts}	1.08 ^t	1.06 ^{bac}

*Means with same superscript are not significantly different at $p \leq 0.05$

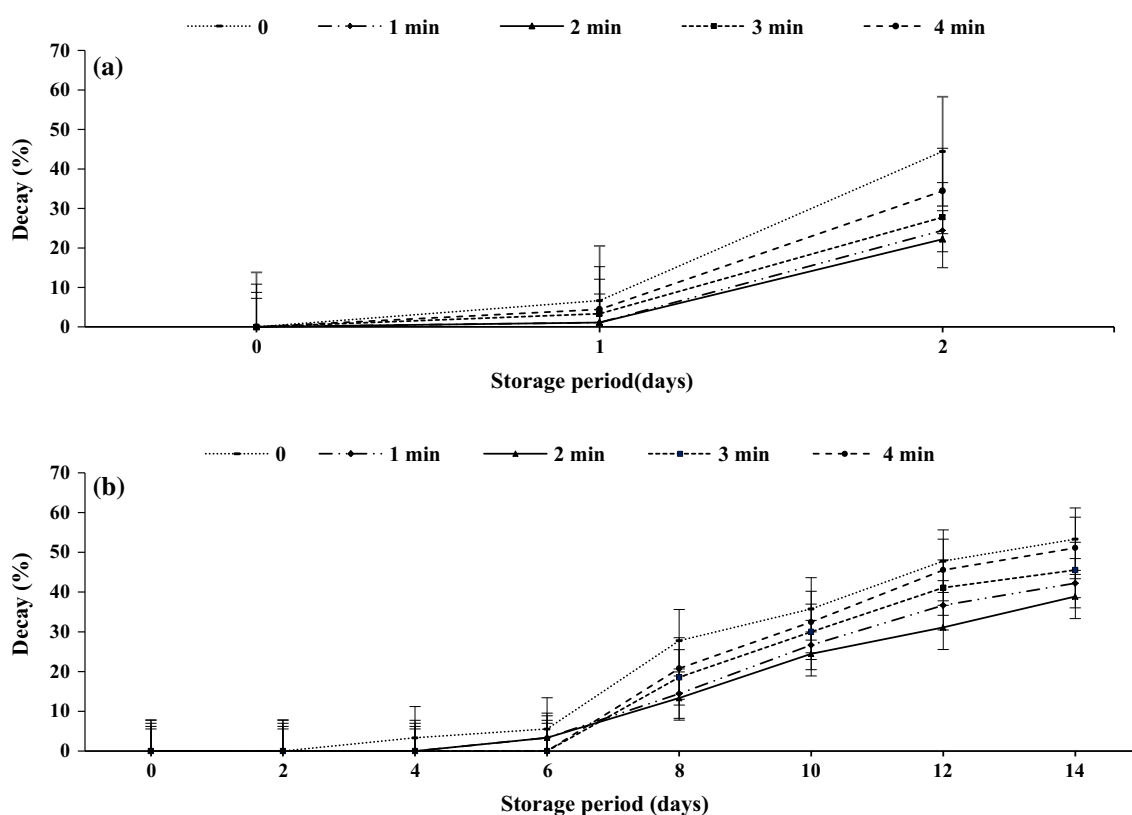


Fig. 1 Influence of aqueous ozone dip treatment on decay (%) in strawberry fruits during **a** ambient and **b** low temperature storage ($n = 3$, vertical bars represent standard deviation)

ozone treated fruits. Lowest decay incidence (38.8%) was observed in fruits exposed to ozone for 2 min and the highest (53.3%) in untreated fruits (Fig. 1) till 14th day of storage. Lower decay in ozone treated strawberry fruits might be due to the antimicrobial action of ozone (Liu et al. 2016). The oxidative nature of ozone interferes with protein synthesis and enzyme activity of the microbial cell and leads to production of free radicals that rupture the cell

membrane leading to lysis of microbial cell (García-Martín et al. 2018).

Ascorbic acid content

We observed a steady decline in the ascorbic acid content of ozone treated as well as untreated ‘Winter Dawn’ strawberry fruits during storage (Table 3). In general, loss in ascorbic acid content was higher in control fruits and at

Table 3 Effect of aqueous ozone on ascorbic acid (mg/100 g) of strawberry fruits cv. ‘Winter Dawn’ during storage

Time of ozone exposure (min)	Ambient (25 ± 2 °C and 45–50% RH)					Low temperature (2 ± 1 °C and 90% RH)					
	Storage period (days)					Storage period (days)					
	0	1	2	0	2	4	6	8	10	12	14
0	52.96 ^{ba}	50.63 ^{bdac}	48.06 ^e	47.36 ^{ebdhagcf}	45.96 ^{gdhigcf}	44.10 ^{jlmhigkf}	43.05 ^{cdemk}	42.46 ^{nlmiko}	38.50 ^{po}	38.50 ^{po}	36.16 ^p
1	51.80 ^{bac}	49.93 ^{ebdc}	49.09 ^{ede}	50.40 ^{bac}	49.23 ^{ebdac}	47.83 ^{ebdagcf}	47.07 ^{ebdhag}	46.66 ^{ebdhigcf}	43.63 ^{njlmhigk}	43.63 ^{njlmhigk}	42 ^{njlmiko}
2	52.96 ^{ba}	51.33 ^{bdac}	49.70 ^{ede}	48.53 ^{ebdacf}	47.60 ^{ebdhagcf}	46.43 ^{ebdhigcf}	45.82 ^{gdhigk}	45 ^{jdihgkf}	42.93 ^{njlmhiko}	42.93 ^{njlmhiko}	41.53 ^{njlmiko}
3	53.20 ^a	51.10 ^{ebdac}	48.76 ^e	47.83 ^{ebdagcf}	46.66 ^{ebdhigcf}	45.03 ^{ejldihgkf}	44.10 ^{ejlmhi}	43.63 ^{njlmhigk}	40.13 ^{nmpo}	40.13 ^{nmpo}	38.26 ^{po}
4	53.66 ^a	51.10 ^{ebdac}	48.30 ^{ed}	49.23 ^{ebdac}	47.83 ^{ebdhagcf}	46.20 ^{ejdhigcf}	45.26 ^{gdhigcf}	44.80 ^{ejlmhigkf}	41.06 ^{nlmiko}	41.06 ^{nlmiko}	38.96 ^{mpo}

*Means with same superscript are not significantly different at $p \leq 0.05$

higher ozone exposure time. Ozone treatment of fruits for 2 min exhibited maximum ascorbic acid content (49.70 mg/100 g) under ambient storage conditions whereas lowest value (48.06 mg/100 g) was displayed by control fruits. Under low temperature storage conditions, the lowest ascorbic acid content (36.16 mg/100 g) was exhibited by untreated fruits as compared to fruits subjected to 2 min in aqueous ozone (41.53 mg/100 g) (Table 3). Higher loss of ascorbic acid in untreated fruits could be attributed to its utilization during the process of respiration. Furthermore, owing to the antioxidative nature, ascorbic acid might be involved in scavenging free radicals produced during ozonation of strawberry fruits thereby leading to its reduction (Alothman et al. 2010; Zhang et al. 2011). It was also observed that aqueous ozone dip for longer duration (> 2 min) retained lesser ascorbic acid content in strawberry fruits over other treatments. Longer exposure of strawberry fruits to ozone might have activated the ascorbate oxidase enzyme that is responsible for conversion of ascorbic acid to dehydroascorbic acid thus, causing a greater reduction in ascorbic acid content during storage (Lee and Kader 2000). Negative effects of long term ozone treatment in reducing concentration of ascorbic acid have also been reported by Alothman et al. (2010) in pineapple fruit.

Total phenolic content

Under ambient storage conditions, lowest total phenolic content was recorded in untreated strawberry fruits (200.76 mg GAE/100 g) while the highest content was observed in fruits dipped in ozonated water for 2 min (401.96 mg GAE/100 g). Surprisingly, phenolic content in these fruits was nearly 50% higher as compared to control fruits (Fig. 2). Similarly, under low temperature storage conditions also, maximum total phenolic content (370.32 mg GAE/100 g) was observed in fruits subjected to same treatment and minimum (234.65 mg GAE/100 g) in control fruits. Higher phenolic content in treated strawberries may be due to oxidative stress causing action of ozone that elicits plant defense mechanisms (Hernandez et al. 2007). Further, ozone exposure also activates the phenylalanine ammonia lyase (PAL) enzyme activity that stimulates the phenol production (Alothman et al. 2010). However, this trend was not continuous, as after 6th day of storage, a slow decline in total phenolic content was observed in all treatments, which might be due to their utilization during storage.

Total anthocyanin content

Anthocyanins are the pigments that impart attractive red colour to strawberry fruit. These pigments are also

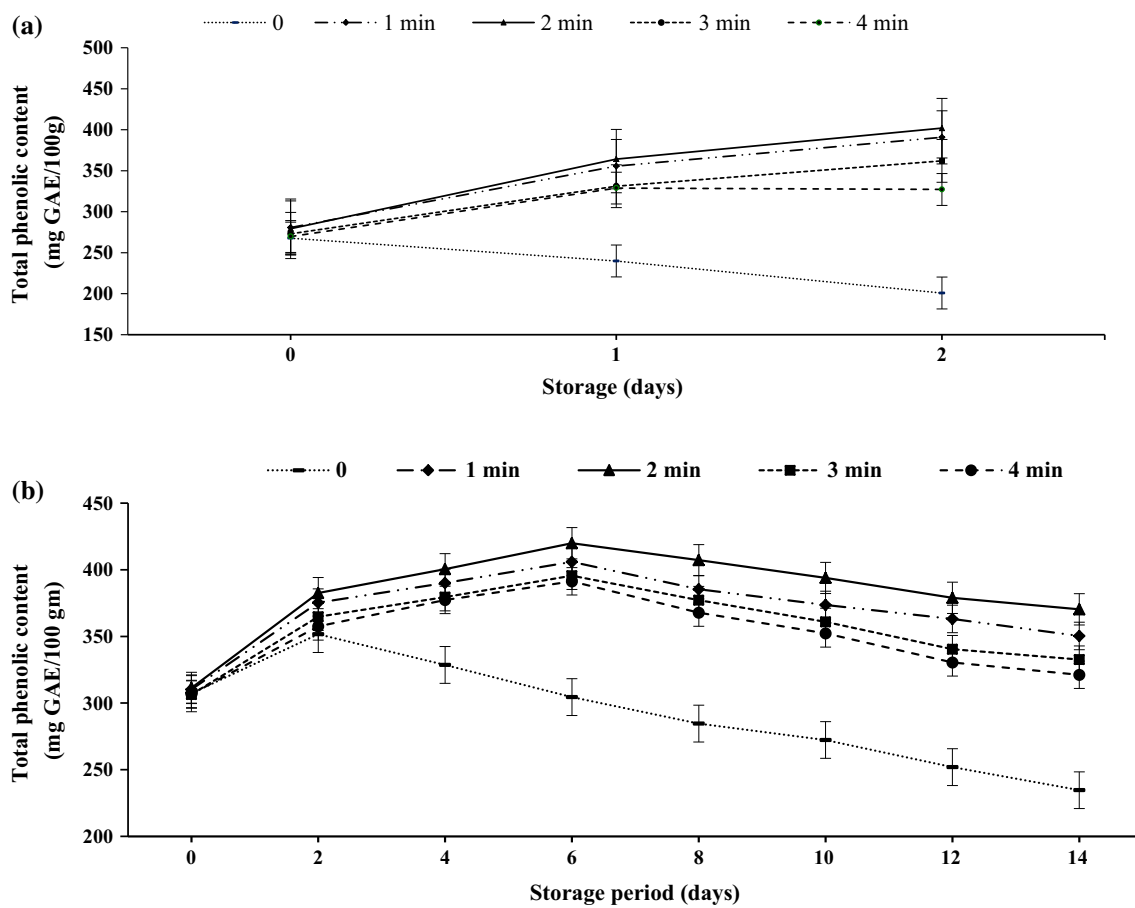


Fig. 2 Changes in total phenolic content (mg GAE/100 g) in strawberry fruits treated with ozonated water and stored under **a** ambient and **b** low temperature conditions ($n = 3$, vertical bars represent standard deviation)

responsible for imparting the antioxidant properties. During the two day storage period under ambient conditions, the lowest anthocyanin content was observed in ‘Winter Dawn’ strawberry fruits which were treated with aqueous ozone for 4 min (368.76 mg/kg) (Fig. 3). The highest anthocyanin content (388.65 mg/kg) on the other hand was observed in fruits exposed to aqueous ozone for 2 min. A similar trend was observed for strawberry fruits stored under low temperature with maximum anthocyanin content (204.76 mg/kg) was recorded in treated fruits and minimum in control fruits (119.96 mg/kg) (Fig. 3). A linear decrease in the anthocyanin content of the fruits was recorded during the storage period but 0.1 ppm ozone treated fruit for 2 min maintained the best fruit colour (Fig. 3). Again, it was really interesting to note that exposure of ‘Winter Dawn’ strawberry fruit to longer duration of ozone could not retain better fruit colour as compared to shorter duration. This could be due to strong oxidizing nature of ozone that results in formation of various intermediate radicals which breakdown the aromatic ring of pigments and form ozonide (Xu et al. 2008). Further, anthocyanins being antioxidative in nature, scavenge

the free radicals produced in the berries following ozone treatment which results in their reduction (Alothman et al. 2010). Our study is well supported by the findings of Tabakoglu and Karaca (2018) who reported no adverse effect of applied ozone on anthocyanin contents of the black mulberry fruits. Therefore, aqueous ozone dip or storage in ozone-enriched atmosphere could be recommended to maintain and improve quality attributes of horticultural commodity.

Conclusion

Effectiveness of ozone application in aqueous solution for reducing decay and maintaining the quality of fruits is dependent on its dose and exposure time. Our study reveals that aqueous ozone can be applied as a sanitizing agent on strawberry fruits for extending postharvest life. Ozone treated ‘Winter Dawn’ fruits showed lower weight loss and higher firmness as compared to control fruits. Also, fruits exhibited lesser change in colour and retained higher phenolic content as compared to untreated fruits. In

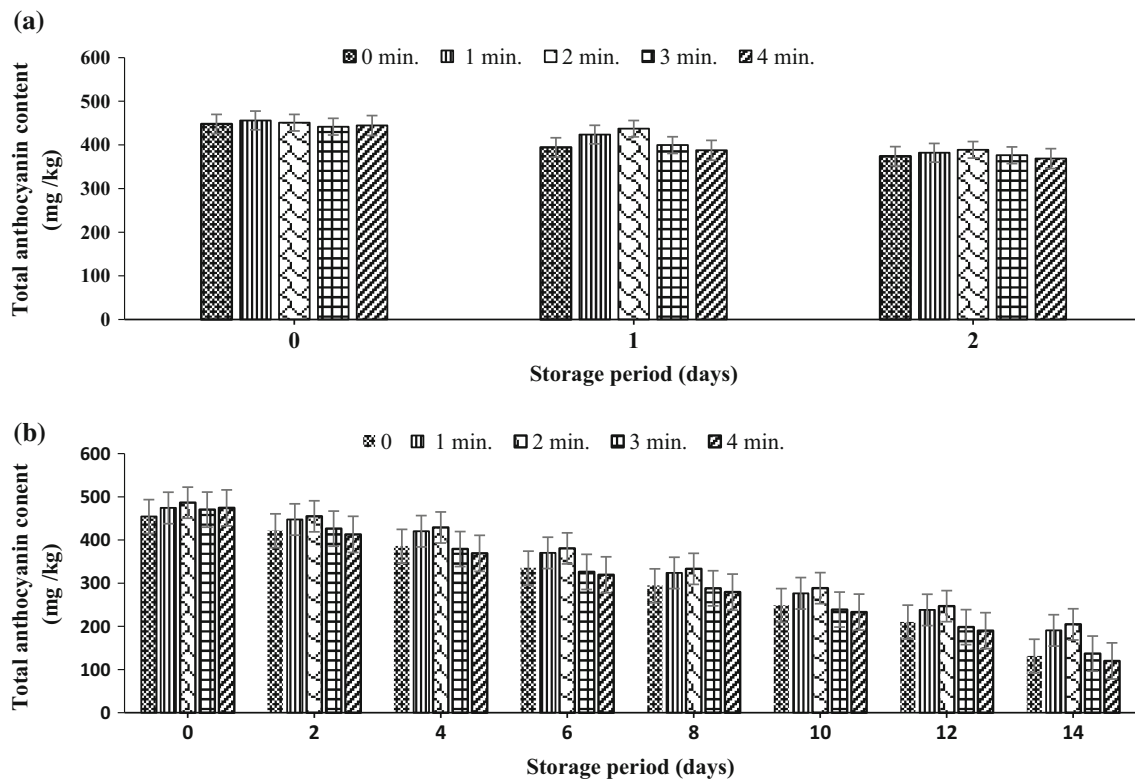


Fig. 3 Influence of time of aqueous ozone treatment on total anthocyanin content (mg/kg) in strawberry fruits during **a** ambient and **b** low temperature storage (n = 3, vertical bars represent standard deviation)

conclusion, the obtained results indicate that treatment of strawberry fruits with 0.1 ppm aqueous ozone for 2 min has a positive influence and can help to preserve fruit quality and control decay up to 14 days at 2 ± 1 °C and 2 days at 25 ± 2 °C. Further, prolonged exposure of strawberry fruits to aqueous ozone may negatively affect the fruit quality. Hence, it is essential to standardize the ozone treatment to a level that does not impair the quality and shelf life of the treated produce.

Compliance with ethical standards

Conflict of interest No potential conflict of interest was reported by the authors.

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