Exposure to ozone reduces postharvest quality loss in red and green chilli peppers

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ABSTRACT

The effect of continuous exposure to ozone at 0.45, 0.9 and 2 µmol mol^{-1} on quality changes during the storage of red and green chilli peppers at 10 °C was investigated. Ozone at 0.45 and 0.9 µmol mol^{-1} reduced disease incidence in red peppers, with no further benefits at 2 µmol mol^{-1}. Ozone at 0.9 µmol mol^{-1} reduced weight loss during storage and improved firmness maintenance. Skin colour was bleached in red peppers exposed to ozone at 2 µmol mol^{-1}, and in green ones at all tested doses. Total phenolic content was not affected by ozone but antioxidant activity was reduced in green chilli peppers exposed to ozone at 2 µmol mol^{-1}, due to lower ascorbic acid content in those samples. Ozone at 0.9 µmol mol^{-1} extended the shelf-life of chilli peppers.

Keywords:

Fresh produce

Microbial contamination

Firmness

Visual quality

Antioxidants

1. Introduction

Chilli peppers' shelf life is limited by both, contamination with microorganism, including human pathogens, e.g. *Escherichia coli* (Cerna-Cortes et al., 2012) and visual and textural quality loss (Nunes, Emond, Rauth, Dea, & Chau, 2009). Chlorine is the most common sanitiser used in the fresh produce industry (Gil, Selma, Lopez-Galvez, & Allende, 2009); however, there is increasing concern about chlorine being over-used and its real efficacy during storage. Thus, the advantages and limitations of numerous alternative methods, e.g. the use of hydrogen peroxide, organic acids, and UV radiation have been reviewed (Ramos, Miller, Brandao, Teixeira, & Silva, 2013).

The interest in using ozone as a postharvest treatment of fruit and vegetables has recently increased (Miller, Silva, & Brandao, 2013; Horvitz & Cantalejo, 2014; Glowacz, Colgan, & Rees, 2015a) due to its potential to reduce microbial contamination of the produce, without any chemical residues being left (Khadre, Yousef, & Kim, 2001), and having no adverse effect on the product's quality, if used at the proper dose.

A number of authors (Ketteringham, Gausseres, James, & James, 2006; Alexandre, Santos-Pedro, Brandao, & Silva, 2011; Horvitz & Cantalejo, 2012; Alexopoulos et al., 2013; Glowacz, Colgan, & Rees, 2015b) studied the efficacy of ozone in reducing microbial counts on bell peppers, and a few (Horvitz & Cantalejo, 2010a, b, 2012; Glowacz, Colgan, & Rees, 2015b) also assessed its effect on physicochemical properties. However, the information on the effects of ozone treatment on the postharvest quality of chilli peppers is scarce (Chitravathi, Chauhan, Raju, & Madhukar, 2015) and requires further investigation.

Microbial counts were found to be reduced on fresh-cut red bell peppers treated with gaseous ozone at 0.7 μmol mol^{-1} for 1-5 minutes prior to storage (Horvitz & Cantalejo, 2010b, 2012) and on whole red bell peppers continuously exposed to ozone at 0.1 and 0.3 μmol mol^{-1} (Glowacz, Colgan, & Rees, 2015b) during a 14-day storage period and with a more pronounced effect at the higher dose.

The efficacy of aqueous ozone in reducing microbial loads on fresh-cut red (Alexandre, Santos-Pedro, Brandao, & Silva, 2011) and whole green (Alexopoulos et al., 2013) bell peppers was found to increase with increasing dose of ozone. However, Ketteringham, Gausseres, James, and James (2006) and Horvitz and Cantalejo (2010a) did not find positive effects of aqueous ozone treatment of fresh-cut peppers. Cut surfaces promote leaching of organic matter that reacts with ozone, thereby reducing its efficiency as an antimicrobial agent. Thus, it has been suggested to treat whole rather than pre-cut peppers.

In a recent study (Chitravathi, Chauhan, Raju, & Madhukar, 2015), aqueous ozone treatment at 30 μmol mol^{-1} for 10 min prior to storage reduced microbial counts on chilli peppers during subsequent storage at 8 °C. However, and to the best of our knowledge, there is no information in the literature on the effects of continuous exposure to gaseous ozone on the postharvest quality of chilli peppers. In the previous study (Glowacz, Colgan, & Rees, 2015b) no signs of rotting were observed in bell peppers continuously exposed to ozone at 0.3 μmol mol^{-1}, while the growth of fungi on the stem and peduncle was observed in 8.3% and 25% of the fruit continuously exposed to ozone at 0.1 μmol mol^{-1} and untreated control, respectively. The objective of this study was to investigate the effects of continuous exposure to ozone at 0.45, 0.9 and 2 μmol mol^{-1} on disease incidence and the physicochemical characteristics of red and green chilli peppers.

2. Materials and methods

2.1. Plant material and handling

Free from visible defects red and green chilli peppers (*Capsicum annuum* L.), varieties Serenade and Jalapeno, respectively, were supplied by Barfoots of Botley Ltd, West Sussex, UK.

Experiment design and ozone fumigation system set up was previously described by Glowacz, Colgan, and Rees (2015b). Fruit were kept at 10 ± 1°C, and continuously exposed to ozone at approximately 0.45 ± 0.10, 0.9 ± 0.10 and 2 ± 0.20 µmol mol^{-1}, using FPTU ozone generators (Onnic International, UK). Control chilli peppers were stored under air. Air was circulated to ensure even distribution of ozone and gas concentration was monitored periodically, on the sampling day before taking the produce out from the containers for subsequent assessment, with an L-106 Ozone Monitor (2B Technologies, US). Relative humidity inside the containers was maintained at 90 ± 3% and monitored using humidity loggers (Lascar Electronics Ltd, UK). Produce quality, i.e. weight loss, visual quality (signs of rotting, shrivelling, stem browning, skin colour), firmness, content of sugars, bioactive compounds and antioxidant activity, was assessed on arrival and after 7, 10 and 14 days of storage.

2.2. Measurements

2.2.1. Weight loss

Weight loss (%) was determined by comparing the weight of the fruit on the sampling day with their initial weight determined on day 0.

2.2.2. Visual quality and firmness

Rotting, shrivelling and stem browning were recorded as a score (0 or 1 - no/signs of rotting, shrivelling and stem browning, respectively). The number of fruit with defects was recorded and calculated as % of the assessed sample population (30 chilli peppers from each replicate). Skin colour and fruit firmness were determined using a Minolta CR-400 chroma meter (Minolta, Japan) and a TA.XT plus Texture Analyser (Stable Micro Systems, UK), respectively, as previously described (Glowacz, Colgan, & Rees, 2015b).

2.2.3. Biochemical analyses

Sugars, ascorbic acid (AsA) and total phenolic content were measured by methods given in Glowacz, Colgan, and Rees (2015b), whereas antioxidant activity FRAP (ferric reducing antioxidant power) and the ability of fruit extracts to scavenge DPPH (2,2-diphenyl-1-picrylhydrazyl) free radicals was determined using the method previously described by Ali, Ong, and Forney (2014).

2.3. Statistical analyses

Chilli peppers were organised in 6 replicates of 90 peppers, for each variety. Data are presented as mean values from a fully randomised design. The significance of main effect was established using ANOVA. Tukey's test was used to compare individual treatment values. All statistical analyses were performed using GenStat 17th Edition software (VSN International Ltd, UK).

3. Results and discussion

3.1. Disease incidence

Red chilli peppers were found to be more prone to rotting, compared to green chilli peppers, in which rots were not observed during the storage period. Signs of rotting (primarily moulds) were observed on red chilli peppers after 7 days of storage on 11.1% and 2.8% of the control samples and those exposed to ozone at 0.45 μmol mol^{-1} whilst no microbial growth was found on those peppers subjected to 0.9 and 2 μmol mol^{-1} gaseous ozone. After 10 days, 16.7% of the control samples showed signs of rotting, whereas disease incidence was significantly reduced to 4.2% in chilli peppers exposed to ozone at 0.45, 0.9 and 2 μmol mol^{-1}, without any difference between doses. Finally, after 14 days of storage, 25% of the control samples where rotted, whilst signs of rotting were observed on 8.3, 8.3 and 16.7% of chilli peppers exposed to ozone at 0.45, 0.9 and 2 μmol mol^{-1}, respectively. Disease incidence was substantially reduced at both 0.45 and 0.9 μmol mol^{-1}. The highest dose of ozone used, probably led to tissue damage, thus facilitating fungal infection, in this way counteracting the beneficial antimicrobial action of ozone.

Reduced disease incidence in peppers exposed to ozone at 0.45 and 0.9 μmol mol^{-1} is in agreement with the results observed by Glowacz, Colgan, and Rees (2015b) and Horvitz and Cantalejo (2010b, 2012), who observed reduced microbial counts on whole red bell peppers continuously exposed to ozone at 0.1 and 0.3 μmol mol^{-1} (Glowacz, Colgan, & Rees, 2015b) and fresh-cut red bell peppers treated with gaseous ozone at 0.7 μmol mol^{-1} for 1-5 minutes prior to storage (Horvitz & Cantalejo, 2010b, 2012), respectively. On the other hand, it is also clear that the dose of ozone has to be appropriately adjusted for each commodity (Forney, 2003) to avoid unwanted tissue damage.

3.2. Weight loss, shrivelling and stem browning

Chilli peppers lost weight over the storage period. The weight loss was lower in both red and green chilli peppers exposed to ozone at 0.9 μmol mol^{-1}; however, this effect was lost after 14 days in ozone-exposed green chilli peppers (Table 1).

Shrivelling and stem browning are both indicators of reduced quality related to the loss of water. The appearance of signs of shrivelling was delayed in red chilli peppers exposed to ozone at 0.45 and 0.9 μmol mol^{-1}, while stem browning was significantly reduced only in samples exposed to ozone at 0.9 μmol mol^{-1} up to 10 days of storage (Table 1). Green chilli peppers were more susceptible to shrivelling than red ones. Shrivelling was reduced up to 10 days of storage in green chilli peppers exposed to ozone at 0.9 μmol mol^{-1} (Table 1). Increasing the dose from 0.9 to 2 μmol mol^{-1}, enhanced shrivelling, this suggests that the dose of ozone at 2 μmol mol^{-1} was too high, and reduced visual quality of the produce.

Reduced weight loss has previously been observed in kiwi continuously exposed to ozone at 0.3 μmol mol^{-1} for 5 months (Minas et al., 2012), cucumbers and courgettes continuously exposed to ozone at 0.1 μmol mol^{-1} for 17 days (Glowacz, Colgan, & Rees, 2015b) and chilli peppers treated with aqueous ozone at 30 μmol mol^{-1} for 10 min (Chitravathi, Chauhan, Raju, & Madhukar, 2015). Water loss from chilli peppers occurs primarily through the cuticle (Kissinger et al., 2005), thus the amount of water loss during storage could be affected by its thickness and composition (Parsons et al., 2013; Lara, Belge, & Goulao, 2014). Thick cuticle makes the produce less susceptible to damage by preventing the epidermal tissues from ozone action (Ali et al., 2014). The mechanism of ozone action in chilli peppers may involve its effect, via reactive oxygen species (ROS) (Kangasjarvi, Jaspers, & Kollist, 2005), on the activity of lipoxygenase (LOX), which could lead to reduced membrane damage and skin surface cracking, and reduced water loss (Lara, Belge, & Goulao, 2014).

3.3. Colour

Exposure to ozone at 0.45 and 0.9 μmol mol^{-1} had no relevant impact on colour characteristics of red chilli peppers, while in samples exposed to ozone at 2 μmol mol^{-1}, a^* (33.94 ± 0.29) and b^* (16.65 ± 0.27) values were significantly higher than control samples (a^* 31.00 ± 0.35; b^* 15.92 ± 0.41) after 14 days, i.e. chilli peppers were more red/yellow, suggesting colour bleaching by the high dose of ozone. Hue angle, however, was not affected, being in the range of 26-27 for all treatments.

In contrast, exposure to ozone even at the lowest doses of 0.45 and 0.9 μmol mol^{-1} affected the colour of green chilli peppers, *i.e.* they became brighter/lighter (higher L^* value) with ozone treatment, especially after 14 days of storage (L^* 35.00 ± 0.52, 36.06 ± 0.37, 36.31 ± 0.40, and 36.65 ± 0.47 in control samples and those exposed to ozone at 0.45, 0.9 and 2 μmol mol^{-1}, respectively), suggesting that characteristic dark green colour could be bleached by ozone, due to accelerated chlorophyll degradation. This was further confirmed by hue angle being significantly reduced from 132.2 ± 0.6 in control to 128.6 ± 0.3, 129.4 ± 0.2, 127.8 ± 0.2 in chilli peppers exposed to ozone at 0.45, 0.9 and 2 μmol mol^{-1}. However, these differences were not always visually obvious.

It has previously been reported that continuous exposure to ozone at 0.1-0.3 μmol mol^{-1} had no significant effect on skin colour of red bell peppers (Glowacz, Colgan, & Rees, 2015b). Similarly, the colour was not affected in minimally processed peppers treated with ozone at 0.7 μmol mol^{-1} for up to 5 min (Horvitz & Cantalejo, 2012). The findings from this study, however suggest that: i) there is a threshold in the ozone dose, i.e. continuous exposure at above 1 μmol mol^{-1}, that would affect colour of red chilli peppers; ii) green chilli peppers are more sensitive to ozone than red ones.

3.4. Firmness

Both, green and red chilli peppers showed softening during storage (Table 2). In red chilli peppers firmness was reduced during storage in all treatments, but was less pronounced in samples exposed to ozone, being highest at 0.9 μmol mol^{-1}. In the case of green chilli peppers no significant difference was observed between control samples and those exposed to ozone, regardless of the dose used. However, firmness maintenance seemed to be improved in ozone exposed chilli peppers at day 10, i.e. the loss of firmness was reduced/delayed. Improved firmness maintenance in ozone exposed chilli peppers is in agreement with findings previously reported for chilli peppers exposed to ozone at 30 μmol mol^{-1} for 10 min prior to storage at 8 °C (Chitravathi, Chauhan, Raju, & Madhukar, 2015).

It has been suggested that in the commodities, where the exposure to ozone can significantly reduce water loss during storage, firmness maintenance would be improved (Glowacz, Colgan, & Rees, 2015b) and in agreement with this, weight loss was also found to be reduced in ozone exposed chilli peppers (Chitravathi, Chauhan, Raju, & Madhukar, 2015).

Several studies have already reported better firmness retention in ozone exposed fruit, e.g. in cucumbers and courgettes continuously exposed to ozone at 0.1 μmol mol^{-1} (Glowacz, Colgan, & Rees, 2015b), in tomatoes cyclically exposed to ozone at 4 μmol mol^{-1} for 30 min every 3 h (Aguayo, Escalona, & Artes, 2006), and continuously exposed to ozone at 0.05 μmol mol^{-1} and 1 μmol mol^{-1} (Tzortzakis, Borland, Singleton, & Barnes, 2007).

3.5. Chemical quality characteristics

3.5.1. Sugars

Exposure of red chilli peppers to ozone at 0.9 μmol mol^{-1} led to significantly higher content of fructose compared with control samples (Table 3) while the content of glucose was not affected. At higher dose, i.e. 2

μmol mol^{-1}, the content of glucose was reduced which could be associated with increased respiration due to tissue damage – the dose of ozone being too high. On the other hand, except the fact that sugar content increased over the storage period in all treatments (Table 3) possibly due to ripening, there was no clear pattern of response in case of green chilli peppers.

3.5.2. Ascorbic acid content

In red chilli peppers the content of AsA was not affected until the end of the storage period, when AsA content was significantly increased in chilli peppers exposed to ozone at 0.9 and 2 μmol mol^{-1} (Table 4). On the other hand, the content of DHA - oxidised form of AsA, was found to be reduced in those samples. The highest content of DHA, which is often considered as an indication of stress was observed in the control samples (Table 4), however care is needed, as DHA can undergo further conversion, e.g. an irreversible hydrolysis to 2,3-diketogulonic acid.

In green chilli peppers, no significant differences among the treatments were observed until the end of the storage period (day 14), when AsA content decreased and DHA content increased in peppers exposed to ozone at 2 μmol mol^{-1} (Table 5), which suggests that these samples were under excess oxidative stress and nutritional quality was reduced. Exposure to ozone at 2 μmol mol^{-1} probably led to an increase in ROS, which then needed to be scavenged by AsA. Plant cells have the capability to reduce the damage caused by ROS using antioxidant enzymes – superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione reductase (GR), catalase (CAT) and metabolites, including AsA and glutathione (GSH) – to transform ROS to less toxic compounds, e.g. water, using AsA as an electron donor (Mittler, 2002). In the reaction catalysed by APX, AsA is changed into DHA. The loss of AsA can be reduced when the activity of dehydroascorbate reductase (DHAR), an enzyme responsible for converting DHA to AsA is increased.

Changes in the content of AsA during 10 days of storage are in agreement with results observed by others, where AsA content was not altered in whole tomatoes cyclically exposed to gaseous ozone at 4 μmol mol^{-1} for 30 min every 3 h (Aguayo, Escalona, & Artes, 2006) or continuously exposed to ozone at 1 μmol mol^{-1} for 6 days (Tzortzakis, Borland, Singleton, & Barnes, 2007). Highest AsA: DHA ratio observed in red chilli peppers exposed to ozone at 0.9 μmol mol^{-1} indicates higher efficiency of AsA-GSH cycle, which is responsible for regeneration of AsA and has been suggested to play a role in extending the shelf-life of fresh produce (Shigenaga, Yamauchi, Funamoto, & Shigyo, 2005).

3.5.3. Total phenolic content

There were no significant differences among the treatments in terms of total phenolic content. At the end of the storage period, however, total phenolic content was slightly but not significantly reduced in chilli peppers exposed to ozone at 2 μmol mol^{-1}. This finding is in agreement with the results observed by Glowacz, Colgan, and Rees (2015b) who did not observe significant differences between red bell peppers exposed to ozone at 0.1 and 0.3 μmol mol^{-1} and control samples. Tzortzakis, Borland, Singleton, and Barnes (2007) also reported that no significant differences were observed between tomatoes exposed to ozone at 1 μmol mol^{-1} and untreated control. The slight decline in total phenolic content at the end of the storage period could be associated with their oxidation by ozone.

3.5.4. Antioxidant activity

Regardless of ozone concentration, antioxidant activity was not affected in red chilli peppers (Table 4). Tzortzakis, Borland, Singleton, and Barnes (2007) also did not observe changes in antioxidant activity in tomatoes exposed to ozone at 1 μmol mol^{-1} for 6 days. In contrast, antioxidant activity was found to be significantly reduced after 14 days in green chilli peppers exposed to ozone at 2 μmol mol^{-1} when compared with control samples while it was not affected at 0.45 and 0.9 μmol mol^{-1} (Table 5), suggesting that green peppers were more sensitive to ozone treatment. Since total phenolic content was not significantly reduced, the observed change in antioxidant activity could be associated with a decline in ascorbic acid content and/or changes in phenolic composition in those samples, presumably due to oxidative stress.

4. Conclusion

Continuous exposure of red chilli peppers to ozone at 0.9 μmol mol^{-1} resulted in significant reduction in disease incidence, reduced weight loss and improved firmness maintenance, while total phenolic content and antioxidant activity were not affected. In green chilli peppers, exposure to ozone at 0.9 μmol mol^{-1} reduced weight loss and shrivelling during storage; firmness maintenance was improved after 10 days of storage. The skin colour was lighter at all tested doses, but the produce was still marketable. The application of ozone at 0.9 μmol mol^{-1} seems to be a feasible solution for reducing quality loss during the storage of both red and green chilli peppers, being more suitable for red chilli peppers.

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Conflict of interest

None

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