

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 $\mu\text{mol mol}^{-1}$ on quality changes during the storage of red and green chilli peppers at 10 °C was investigated. Ozone at 0.45 and 0.9 $\mu\text{mol mol}^{-1}$ reduced disease incidence in red peppers, with no further benefits at 2 $\mu\text{mol mol}^{-1}$. Ozone at 0.9 $\mu\text{mol mol}^{-1}$ reduced weight loss during storage and improved firmness maintenance. Skin colour was bleached in red peppers exposed to ozone at 2 $\mu\text{mol mol}^{-1}$, 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 $\mu\text{mol mol}^{-1}$, due to lower ascorbic acid content in those samples. Ozone at 0.9 $\mu\text{mol mol}^{-1}$ 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 $0.7 \mu\text{mol mol}^{-1}$ 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 $0.3 \mu\text{mol mol}^{-1}$ (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 $30 \mu\text{mol mol}^{-1}$ 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 $0.3 \mu\text{mol mol}^{-1}$, 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 $0.1 \mu\text{mol mol}^{-1}$ 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 $2 \mu\text{mol mol}^{-1}$ on disease incidence and the physicochemical characteristics of red and green chilli peppers.

1 2. Materials and methods

2 2.1. Plant material and handling

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

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

15 2.2. Measurements

16 2.2.1. Weight loss

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

19 2.2.2. Visual quality and firmness

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

25 2.2.3. Biochemical analyses

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

30 2.3. Statistical analyses

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

35 **3. Results and discussion**

36 3.1. Disease incidence

37 Red chilli peppers were found to be more prone to rotting, compared to green chilli peppers, in which
38 rots were not observed during the storage period. Signs of rotting (primarily moulds) were observed on red chilli
39 peppers after 7 days of storage on 11.1% and 2.8% of the control samples and those exposed to ozone at 0.45
40 $\mu\text{mol mol}^{-1}$ whilst no microbial growth was found on those peppers subjected to 0.9 and 2 $\mu\text{mol mol}^{-1}$ gaseous
41 ozone. After 10 days, 16.7% of the control samples showed signs of rotting, whereas disease incidence was
42 significantly reduced to 4.2% in chilli peppers exposed to ozone at 0.45, 0.9 and 2 $\mu\text{mol mol}^{-1}$, without any
43 difference between doses. Finally, after 14 days of storage, 25% of the control samples where rotted, whilst
44 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 $\mu\text{mol mol}^{-1}$
45 mol^{-1} , respectively. Disease incidence was substantially reduced at both 0.45 and 0.9 $\mu\text{mol mol}^{-1}$. The highest
46 dose of ozone used, probably led to tissue damage, thus facilitating fungal infection, in this way counteracting
47 the beneficial antimicrobial action of ozone.

48 Reduced disease incidence in peppers exposed to ozone at 0.45 and 0.9 $\mu\text{mol mol}^{-1}$ is in agreement
49 with the results observed by Glowacz, Colgan, and Rees (2015b) and Horvitz and Cantalejo (2010b, 2012), who
50 observed reduced microbial counts on whole red bell peppers continuously exposed to ozone at 0.1 and 0.3
51 $\mu\text{mol mol}^{-1}$ (Glowacz, Colgan, & Rees, 2015b) and fresh-cut red bell peppers treated with gaseous ozone at 0.7
52 $\mu\text{mol mol}^{-1}$ for 1-5 minutes prior to storage (Horvitz & Cantalejo, 2010b, 2012), respectively. On the other hand,
53 it is also clear that the dose of ozone has to be appropriately adjusted for each commodity (Forney, 2003) to
54 avoid unwanted tissue damage.

55 3.2. Weight loss, shrivelling and stem browning

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

59 Shrivelling and stem browning are both indicators of reduced quality related to the loss of water. The
60 appearance of signs of shrivelling was delayed in red chilli peppers exposed to ozone at 0.45 and 0.9 $\mu\text{mol mol}^{-1}$
61 ¹, while stem browning was significantly reduced only in samples exposed to ozone at 0.9 $\mu\text{mol mol}^{-1}$ up to 10
62 days of storage (Table 1). Green chilli peppers were more susceptible to shrivelling than red ones. Shrivelling
63 was reduced up to 10 days of storage in green chilli peppers exposed to ozone at 0.9 $\mu\text{mol mol}^{-1}$ (Table 1).
64 Increasing the dose from 0.9 to 2 $\mu\text{mol mol}^{-1}$, enhanced shrivelling, this suggests that the dose of ozone at 2
65 $\mu\text{mol mol}^{-1}$ was too high, and reduced visual quality of the produce.

66 Reduced weight loss has previously been observed in kiwi continuously exposed to ozone at 0.3 μmol
67 mol^{-1} for 5 months (Minas et al., 2012), cucumbers and courgettes continuously exposed to ozone at 0.1 μmol
68 mol^{-1} for 17 days (Glowacz, Colgan, & Rees, 2015b) and chilli peppers treated with aqueous ozone at 30 μmol
69 mol^{-1} for 10 min (Chitravathi, Chauhan, Raju, & Madhukar, 2015). Water loss from chilli peppers occurs
70 primarily through the cuticle (Kissinger et al., 2005), thus the amount of water loss during storage could be
71 affected by its thickness and composition (Parsons et al., 2013; Lara, Belge, & Goulao, 2014). Thick cuticle
72 makes the produce less susceptible to damage by preventing the epidermal tissues from ozone action (Ali et al.,
73 2014). The mechanism of ozone action in chilli peppers may involve its effect, via reactive oxygen species
74 (ROS) (Kangasjarvi, Jaspers, & Kollist, 2005), on the activity of lipoxygenase (LOX), which could lead to
75 reduced membrane damage and skin surface cracking, and reduced water loss (Lara, Belge, & Goulao, 2014).

76 3.3. Colour

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

82 In contrast, exposure to ozone even at the lowest doses of 0.45 and 0.9 $\mu\text{mol mol}^{-1}$ affected the colour
83 of green chilli peppers, i.e. they became brighter/lighter (higher L^* value) with ozone treatment, especially after
84 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
85 exposed to ozone at 0.45, 0.9 and 2 $\mu\text{mol mol}^{-1}$, respectively), suggesting that characteristic dark green colour
86 could be bleached by ozone, due to accelerated chlorophyll degradation. This was further confirmed by hue
87 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

88 peppers exposed to ozone at 0.45, 0.9 and 2 $\mu\text{mol mol}^{-1}$. However, these differences were not always visually
89 obvious.

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

96 3.4. Firmness

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

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

109 Several studies have already reported better firmness retention in ozone exposed fruit, e.g. in
110 cucumbers and courgettes continuously exposed to ozone at 0.1 $\mu\text{mol mol}^{-1}$ (Glowacz, Colgan, & Rees, 2015b),
111 in tomatoes cyclically exposed to ozone at 4 $\mu\text{mol mol}^{-1}$ for 30 min every 3 h (Aguayo, Escalona, & Artes,
112 2006), and continuously exposed to ozone at 0.05 $\mu\text{mol mol}^{-1}$ and 1 $\mu\text{mol mol}^{-1}$ (Tzortzakis, Borland, Singleton,
113 & Barnes, 2007).

114 3.5. Chemical quality characteristics

115 3.5.1. Sugars

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

118 $\mu\text{mol mol}^{-1}$, the content of glucose was reduced which could be associated with increased respiration due to
119 tissue damage – the dose of ozone being too high. On the other hand, except the fact that sugar content increased
120 over the storage period in all treatments (Table 3) possibly due to ripening, there was no clear pattern of
121 response in case of green chilli peppers.

122 3.5.2. Ascorbic acid content

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

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

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

146 3.5.3. Total phenolic content

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

155 3.5.4. Antioxidant activity

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

164 **4. Conclusion**

165 Continuous exposure of red chilli peppers to ozone at 0.9 $\mu\text{mol mol}^{-1}$ resulted in significant reduction
166 in disease incidence, reduced weight loss and improved firmness maintenance, while total phenolic content and
167 antioxidant activity were not affected. In green chilli peppers, exposure to ozone at 0.9 $\mu\text{mol mol}^{-1}$ reduced
168 weight loss and shrivelling during storage; firmness maintenance was improved after 10 days of storage. The
169 skin colour was lighter at all tested doses, but the produce was still marketable. The application of ozone at 0.9
170 $\mu\text{mol mol}^{-1}$ seems to be a feasible solution for reducing quality loss during the storage of both red and green
171 chilli peppers, being more suitable for red chilli peppers.

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174 the Innovate UK project on the use of ozone to extend the storage life of fresh produce.

175 **Conflict of interest**

176 None

177 **References**

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