Application of an environmentally friendly preventive measure for the preservation of fresh vegetables

Simona Paulikienė¹ · Algirdas Raila¹ · Renata Žvirdauskienė^{2,3} · Egidijus Zvicevičius¹

Revised: 21 February 2019 / Accepted: 6 March 2019 / Published online: 20 March 2019
© Association of Food Scientists & Technologists (India) 2019

Abstract Vegetables are an important source of vitamins and minerals and are one of the most perishable products. Fresh vegetables need to be cleaned, sorted, washed and dried before entering distribution and trading systems. The quality of the product and the effect of the environment during preparation are very important. One method of stabilizing vegetable quality is the application of ozone, which is a strong disinfectant used to inactivate microorganisms. Ozone, when in contact with a product surface, does not leave any harmful by-products. This research aims to optimize carrot treatment with ozonated water and evaluate its effect and efficiency on the storage of products. A constant concentration of $1.53 \pm 0.09 \text{ mg L}^{-1}$ ozone in water was used, and processing times of 5, 10, 15 and 20 min were applied. The carrots' weight and changes to the mould population on the carrots' surface during storage were observed after treatment with ozonated water. A

decrease in mycological contamination on washable vegetable surfaces occurred within the first 5 min, and longer processing times (more than 5 min) did not influence the efficacy of the treatment. Thus, using ozonated water at an ozone concentration of $1.53 \pm 0.09 \text{ mg L}^{-1}$ reduced the mould population by up to 99.99% and could also prolong carrot storage duration up to 1.7 times. Treatment of carrots with ozonated water had minimal impact on the carrots' weight.

Keywords Ozone · Ozonated water · Vegetables · Carrot · Mould populations

Introduction

According to studies by Pavia et al. (2006) and Pérez-Jiménez and Saura-Calixto (2015), the human body requires fresh vegetables and fruits. Fruits and vegetables are essential components of the human diet, and there is considerable evidence for the health and nutritional benefits associated with their consumption (Ramos et al. 2013). Recently, the supply of rinsed fresh agricultural products has been increasing worldwide. However, fruits and vegetables are among the most perishable foods on the market. Clean products can be seen on store shelves; these vegetables are washed during preparation prior to distribution to the retail network. Therefore, to reduce risks to consumers, chemicals such as chlorine are widely used in the fresh fruit and vegetable industry to reduce microbial contamination and to control pathogens (Ramos et al. 2013; Shankar et al. 2014). When traditional disinfectants are used for rinsing, chemical by-products are produced such as trihalomethanes (THM), haloacetic acids (HAAs) and other chemical compounds, which are returned to sewage

✉ Simona Paulikienė
simona.paulikienel@vdu.lt

Algirdas Raila
algirdas.raila@vdu.lt

Renata Žvirdauskienė
renata.zvirdauskiene@lammc.lt;
renata.zvirdauskiene@ktu.lt

Egidijus Zvicevičius
egidijus.zvicevicius@vdu.lt

¹ Institute of Energy and Biotechnology Engineering, Faculty of Agricultural Engineering, Vytautas Magnus University, Studentų str. 15, Akademija, 53362 Kaunas r., Lithuania

² Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry, Instituto 1, Akademija, Kėdainiai r., Lithuania

³ Faculty of Chemical Technology, Kaunas University of Technology, Radvilėnų Rd. 19, 50254 Kaunas, Lithuania

systems and therefore to the environment. These compounds can adversely affect human and environmental safety (Horvitz and Cantalejo 2014; Pascual et al. 2007). Chemical toxins are typically stable, and therefore, they accumulate in the environment and are then reintroduced to agricultural products and food. These chemicals accumulate in living organisms as well, and their concentrations increase rapidly in the food chain (Pandey et al. 2014).

From the product preservation point of view, the preserved quality of a stored product is not the only aspect that is important; the harmfulness to and the effect on the environment of substances used to extend the time of safe storage are also relevant. The use of certain methods of conservation when preparing products for longer storage is unavoidable; however, the processing costs can be reduced by the use of preventative measures. To safely maintain high product quality for extended periods of time, it is necessary to create storage conditions that are unfavorable to or suppress the activity of microorganisms but are not destructive to the products. At the same time, appropriate environments should be used to create the required microclimatic conditions to reduce energy consumption and the impact on the environment. One solution to this problem is to apply ozone technology as a precautionary measure.

When preparing vegetables for markets, treatment with ozone replaces other chemicals without causing damage to the environment or to the vegetables. Moreover, with proper installation of this technology, the ozone does not affect the human body. The use of low ozone concentrations without any additional chemicals can be effective in eliminating microorganisms from the surface of products (Huyskens-Keil et al. 2011).

Ozone is a very strong disinfectant with an oxidizing potential of 2.07 V, which is 52% higher than that of chlorine (1.36 V), and it affects a wider range of microorganisms (Pascual et al. 2007; Shankar et al. 2014). Ozonated water is generally recognized as safe (GRAS) (Fishburn et al. 2012) and is categorized as an organic chemical disinfectant because, after reaction with microflora, there are no toxic residues left on the surface (Carletti et al. 2013; de Alencar et al. 2011; Pascual et al. 2007). Treatment with ozone inactivate a wide range of viruses, bacteria and moulds (Khadre et al. 2006; Pascual et al. 2007). Ozone can also efficiently inactivate yeasts and fungal spores (Horvitz and Cantalejo 2014).

Much information is available in the literature about the use of ozone and ozonated water for vegetable and fruit rinsing prior to the delivery of produce to consumers. However, there is no information concerning the correct concentration, exposure time and effects on storage for certain types of vegetables. According to Tiwari and Muthukumarappan (2012), ozonation can be used in the

production of vegetables and fruits that are safe, of high quality and less processed, but before treatment with ozone can be used, it is necessary to prepare and define specific processing conditions for each product. Using ozone incorrectly, i.e., using a higher concentration than needed, can lead to unwanted changes in food quality during storage or processing. Therefore, it is necessary to study the optimization of this treatment (Souza et al. 2018). This study selected a variety of carrots that is suitable for long periods of storage. Globally, carrots (*Daucus carota* L.) are one of the most economical and most consumed cultivated vegetables because of the level of carotenoids and other vitamins that they contain (Chen et al. 2016; Hartikainen et al. 2017). Carrots have a thin peel; therefore, protection against pathogens is a key issue. The aim of this article is to determine the effects of ozone on the shelf life of carrots and its impact on mycological contamination.

Materials and methods

Materials

Carrots were planted at the beginning of May and harvested in late September. Mid-sized, uniformly shaped carrots were selected for the study. The mean weight and length of the carrots was 119.83 ± 9.73 g and 185.00 ± 12.00 mm, respectively. The carrots were stored in a climatic chamber at a storage temperature of 1.00 ± 0.50 °C and a relative humidity of $95.00 \pm 2.00\%$.

During the season, carrots are stored in a warehouse in special boxes containing grounds. In this manner, the carrots are stored for up to 8 months (from October to May). Investigations were carried out on randomly chosen months: October, December, January, February, March and May.

Sample preparation

The carrots were divided into six treatments of seven carrots each were individually numbered and weighed. The carrots in the first four treatments were rinsed with tap water and then treated for 5, 10, 15 or 20 min, respectively, with ozonated water. In the fifth treatment, the carrots were rinsed only with tap water, and the sixth treatment contained unrinsed carrots (from storage) as a control.

The treatments were applied as follows:

Sample 1—carrots treated with ozonated water for 5 min;

Sample 2—carrots treated with ozonated water for 10 min;

- Sample 3—carrots treated with ozonated water for 15 min;
 Sample 4—carrots treated with ozonated water for 20 min;
 Sample 5—carrots treated with tap water;
 Sample 6—control (unrinsed) carrots.

Water saturation with ozone gas

The ozone generator OZ-AW-15, with a power output of 2.0 kW, a treated water flow rate of $5.0 \text{ m}^3 \text{ h}^{-1}$ and ozone flow rate of 15.0 g h^{-1} are used to saturate water with ozone gas. An ozone generator hose with an ejector was inserted into the tank with water as the ozone inlet. To obtain an ozone concentration of $1.50\text{--}1.60 \text{ mg L}^{-1}$ in the water, the water was ozonated for 2.5 min.

Treating of samples with ozonated water

Ozonated water was prepared individually for each sample. The ozone concentration in the rinse water for the product was determined prior to treating and after 5, 10, 15 and 20 min. CHEMetrics Chemets test kits were used to determine the ozone concentration of the water.

One carrot was taken from each sample for further microbiological analyses.

Storage

The remaining 6 carrots from each sample were divided into 3 pieces, which were packed into bags with holes and held at one of two temperatures. The first temperature of $3.00 \pm 0.15 \text{ }^\circ\text{C}$ (relative humidity $86.50 \pm 2.00\%$) is the recommended temperature for the storage of most products. The second temperature of $16.00 \pm 1.00 \text{ }^\circ\text{C}$ (relative humidity $41.60 \pm 2.30\%$) was chosen to assess the impact of non-compliance to norms on the qualitative characteristics of the product given the actual conditions of retail stores. The first 6 samples (1.1, 2.1, 3.1, 4.1, 5.1, and 6.1) were held in an incubator at $3.00 \pm 0.50 \text{ }^\circ\text{C}$. The remaining 6 samples (1.2, 2.2, 3.2, 4.2, 5.2, and 6.2) were held in a room at $16.00 \pm 1.00 \text{ }^\circ\text{C}$. The samples were kept for 36 days.

Weight loss

The weight loss of the carrots was determined by weighing the carrots on an electronic scale (SCALTEC SPO 51) on days 1, 8, 20, 28 and 36 of storage.

The variation in the weight of the product (percent) was calculated using the following formula (Ekici et al. 2015):

$$\Delta W_{\%} = \frac{W_1 - W_2}{W_1} \cdot 100\%, \quad (1)$$

where W_1 indicates the initial weight of the product (g) and W_2 indicates the weight of the product at a given temperature (g).

The weight loss (grams per kg) of the initial weight of the product was calculated by:

$$\Delta W = \frac{\Delta W_{\%} \cdot 1000}{100}, \quad (2)$$

where ΔW indicates the change in the weight of the product (g kg^{-1}).

Microbiological tests

Microbiological tests were performed to quantify the population of microorganisms. Carrot specimens were taken from each sample at both temperatures on days 1, 8, 20, and 36.

The microbiological tests were performed in accordance with the ISO standard 21527-1:2008 'Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of yeasts and moulds. Part 1: Colony count technique in products with water activity greater than 0.95'.

Microbiological testing of raw material

One specimen from each carrot (approximately 50.00 g) was aseptically added to a sterile BagLight bag without a filter. Each sample was subsequently blended with 450.00 mL of 0.10% peptone water for 45 s. Serial dilutions of the homogenate (in 0.10% peptone) were surface-plated in duplicate on dichloran-rose Bengal chloramphenicol agar (REF 258710, Difco, USA). The prepared agar plates were incubated for five days at $25.00 \text{ }^\circ\text{C} \pm 1.00 \text{ }^\circ\text{C}$. The colonies were counted; the counts are expressed in colony forming units per mL (CFU mL^{-1}). Moulds were identified using conventional methods based on their macroscopic and microscopic features. The identification was performed according to the methods described by Pitt and Hocking (2009).

The difference in the mould count between treatments (percent) was calculated using the following formula:

$$\Delta N_{\%} = \frac{N_1 - N_2}{N_1} \cdot 100\%, \quad (3)$$

where N_1 indicates the initial mould colony count (CFU mL^{-1}), and N_2 indicates the number of mould colonies observed during the test period or at the final mould count (CFU mL^{-1}).

Statistical analysis

Statistical analyses were conducted using IBM SPSS 24 Statistics software. The differences between the treatment groups were analysed using a one-way analysis of variance ANOVA with Dunnett's *t*-tests. The differences were considered significant at a *P* value of less than 0.05.

The data are summarized as the mean $\pm$ standard deviation. The regression analyses and diagrams were made using Microsoft Excel. The regression analyses were used to show the relation between dependent and independent variables.

Results and discussion

The concentration of 1.50–1.60 mg L⁻¹ ozone was selected to determine the effect of ozone on mycological contamination. This concentration was chosen based on studies conducted by Smilanick (2003), which showed that an ozone concentration of 1.50 mg L⁻¹ in water is sufficient to destroy microorganisms. In addition, Suslow (2004) reports that in clean, potable water free of organic debris and soil particulates, ozone is a highly effective sanitizer at concentrations of 0.50–2.00 ppm (1.00 mg L⁻¹ = 1.00 ppm). In our study, 5.00 L of water was ozonated for 2.5 min, which resulted in a concentration of 1.53 $\pm$ 0.09 mg L⁻¹.

Changes in ozone concentrations in water without carrots, with treated tap water carrots and untreated carrots were researched. The ozone concentration was measured every 5 min for 30 min (initial ozone concentration in water was 1.53 $\pm$ 0.09 mg L⁻¹). It was observed that the ozone concentration of the water mostly dissipated after the first few minutes at 0.30 $\pm$ 0.10 mg L⁻¹ without samples and 0.50 $\pm$ 0.20 mg L⁻¹ with rinsed samples. The unrinsed samples were placed in ozonated water with an ozone concentration of 1.53 $\pm$ 0.09 mg L⁻¹; the ozone concentration in the water stabilized dissipated within 5 min. Studies performed by Baba et al. (2002) and Pierron et al. (2015) showed that the half-life of ozone in distilled water is 20–30 min. The breakdown of ozone depends on the presence of free radicals in the water as well as on the water temperature and pH.

Figure 1 shows the data for 1 year. A model of carrot mould populations showed that during the storage season, the mould contamination of carrots increased logarithmically. The regression coefficient was 0.96, indicating that the resulting model was robust for these data. A significant (*P* < 0.05) increase in the mould population was observed among carrots tested in different months ($D_{\text{Dunnett}} = 3.96 \cdot 10^4$ CFU mL⁻¹). However, the effect of ozonated water was not significant between the performed tests, and for all

samples, the mould population on the carrots was greater than 99.90%. Because the variability in carrot mould populations among months is high, further discussion is limited to randomly selected carrot data from March.

The results of the microbiological tests and variation in the carrot weight on selected days (1, 8, 20, 28 and 36 of storage) were used for the quality assessment. Table 1 shows the growth of mould on carrots. A comparison between the control carrots and the treated carrots was conducted (ANOVA with Dunnett's control post hoc tests, with significance set at 0.05) on days 1, 8, 20, 36 of storage. The differences (*P* < 0.05) in the growth of mould populations among different treatment groups and changes in the mould population during the storage of carrots are also shown; significant differences are indicated by different letters.

The carrot treatment with ozonated water (1.53 $\pm$ 0.09 mg L⁻¹) affected the number of mould colonies; mould populations decreased compared with the control (unrinsed) carrots as well as the carrots that was treated with tap water. Treating the carrots with ozonated water inactivated 99.96 $\pm$ 0.02% of moulds. This is consistent with the results of Gonçalves (2009) and da Silva et al. (1998), who report that up to 99.90% of pesticides and microorganisms can be destroyed with the use of ozone. Pascual et al. (2007) also reports that by using ozone with a concentration of 0.48 mg min⁻¹ L⁻¹, there was a reduction in microorganisms of 3 log units (99.90%). Naito and Takahara (2006) reported that by using ozonated water (4.90% volume/volume, 0.50 L min⁻¹ for 5 min) on salad, the microorganism load decreased by 1.5–1.9 log units. When carrots were treated for 30 min using an ozone injection rate of 5.00/g/h, the number of microorganisms decreased threefold (Naito and Takahara 2006). When applying an ozone concentration of 300.00 or 1000.00 nL L⁻¹ for 1, 2, or 4 days, Priyanka et al. (2014) stated that the lower concentration was more effective in reducing mould populations for approximately 8 weeks after treatment, and the highest concentration had no positive effect.

Compared with the control group at 3.00 $\pm$ 0.15 °C, there is a significant difference between days 1 and 20 within each treated group, whereas on days 8 and 36, a significant difference is only seen in the groups treated with ozonated water. At 16.00 $\pm$ 1.00 °C, all treated samples of carrots had a significant substantial difference in mould populations compared with the control carrots. In every treatment, the carrot mould population was reduced.

There were no significant differences in the mean number of mould colonies between carrots treated with ozonated water for different durations, i.e., 5, 10, 15 and 20 min. Therefore, treating a product with ozonated water for more than 5 min is not necessary because

Fig. 1 Relation between the growth of mould populations on carrots and the period of storage in the control carrots (from October to May). The results are significant at the $P < 0.05$ level (one-way ANOVA with a Dunnett test)

microorganisms are affected during the first few minutes. A 5 min treatment is sufficient for most moulds. According to Smilanick (2003), at an ozone concentration of 1.50 mg L^{-1} , most moulds that are typically found on fruits and vegetables do not survive for more than 3 min. Further studies should examine shorter treating durations for carrots.

The level of mould contamination on the carrots treated with tap water was $94.93 \pm 2.11\%$ higher than on the carrots treated with ozonated water.

Long-term storage had an impact on the growth of the mould population. A steady increase in the mould colony count was observed during microbiological tests on days 8, 20 and 36 of storage.

Changes in the mould population on carrots that were treated for different times (Table 1) and stored at $3.00 \pm 0.15^\circ\text{C}$ were lower than for carrots that were treated with only tap water. The results showed that treating for more than 5 min was not necessary. Additionally, in practice it is not expedient to use longer treatment because of the higher resource use. Therefore, further discussion of Table 1 will only consider the 5 min treatment on 8, 20, 36 days.

On day 8, after an initial treatment with ozonated water, the mould population on ozonated carrots was less apparent compared with carrots treated with tap water or the control carrots. The increase in the average population of moulds on carrots treated with ozonated water was 97.19% lower than on the carrots treated with tap water and 99.95% lower than the population of moulds on the control carrots. This can be explained by the fact that the mould population is

not only reduced but also the growth of the remaining moulds were suppressed for a certain period of time.

Later on the other days (on days 20 and 36) mould population recovery took place, and moulds resumed their growth processes. The largest change in the mould population on the carrots treated with ozonated water occurred on day 20, when the count increased by 86.51% compared with day 8. The growth of the mould population on carrots treated with ozonated water was the highest during this period. From day 20 to day 36 in carrots treated with ozonated water, the growth of the mould population was 53.09%, compared with carrots treated with tap water (57.87%) and control carrots (90.02%).

The increase in the mould population on carrots treated with ozonated water, on carrots treated with tap water and on the control carrots occurred because the growth and activity of mould continues during storage and is dependent upon the storage conditions (Fernández-Cruz et al. 2010). This can be seen in Table 1 (samples 1.2–6.2), which shows a more rapid growth of mould colonies on the carrots stored at $16.00 \pm 1.00^\circ\text{C}$ for 36 days. Because the temperature was considerably higher, the conditions were more favourable for mould growth. The mould colony count increased considerably in carrots treated with ozonated water (164.58 times) and tap water (89.08 times) by day 8. Later, the growth of the mold population increases proportionally, but is not as high as in the first 8 days.

The increase in the mould colony count on the carrots treated with ozonated water and stored at $16.00 \pm 1.00^\circ\text{C}$ was highest at the end of the storage (day 36). The growth of the mould population on these carrots was 70.94% higher than on carrots treated with tap water and 59.97%

Table 1 Total population of the moulds on carrots stored for 36 days

Treatment	Moulds colony count CFU mL ⁻¹				<i>D</i> _{Dunnett}			
	Day 1	Day 8	Day 20	Day 36				
3.0 ± 0.15 °C, Relative humidity—86.5 ± 2.0%								
1.1 Treated with ozonated water for 5 min	2.08 · 10 ² ± 3.78 · 10 ¹	a* a 4.18 · 10 ² ± 7.84 · 10 ¹	a* a 3.10 · 10 ³ ± 2.12 · 10 ²	a* b 6.61 · 10 ³ ± 3.49 · 10 ²	a* c			← 2.96 · 10 ²
2.1 Treated with ozonated water for 10 min	2.98 · 10 ² ± 3.36 · 10 ¹	a* a 6.03 · 10 ² ± 8.72 · 10 ¹	a* a 3.38 · 10 ³ ± 2.69 · 10 ²	a* b 8.02 · 10 ³ ± 3.84 · 10 ²	a* c			← 3.39 · 10 ²
3.1 Treated with ozonated water for 15 min	1.68 · 10 ² ± 3.49 · 10 ¹	a* a 9.60 · 10 ² ± 5.94 · 10 ¹	b* a 5.55 · 10 ³ ± 3.22 · 10 ²	ab* b 9.16 · 10 ³ ± 4.70 · 10 ²	a* c			← 4.07 · 10 ²
4.1 Treated with ozonated water for 20 min	2.73 · 10 ² ± 4.45 · 10 ¹	a* a 8.88 · 10 ² ± 8.52 · 10 ¹	a* b 5.02 · 10 ³ ± 3.05 · 10 ²	ab* c 9.64 · 10 ³ ± 5.44 · 10 ²	a* d			← 4.48 · 10 ²
5.1 Treated with tap water	3.98 · 10 ³ ± 3.49 · 10 ²	b* a 1.49 · 10 ⁴ ± 8.92 · 10 ²	b b 3.53 · 10 ⁴ ± 2.06 · 0 ³	b* c 1.14 · 10 ⁵ ± 3.12 · 10 ³	b d			← 2.14 · 10 ³
6.1 Control carrots	5.23 · 10 ⁵ ± 1.96 · 10 ⁴	c a 8.35 · 10 ⁵ ± 3.20 · 10 ⁴	c b 5.79 · 10 ⁶ ± 2.52 · 0 ⁵	c c 5.80 · 10 ⁷ ± 1.48 · 10 ⁶	c d			← 9.07 · 10 ⁵
<i>D</i> _{Dunnett}	3.39 · 10 ³ ↑	1.20 · 10 ⁴ ↑	3.19 · 10 ⁴ ↑	1.05 · 10 ⁵ ↑				
16.0 ± 1.0 °C, Relative humidity—41.6 ± 2.3%								
1.2 Treated with ozonated water for 5 min	2.08 · 10 ² ± 3.78 · 10 ¹	a* a 3.43 · 10 ⁴ ± 2.39 · 10 ³	a* b 1.05 · 10 ⁵ ± 6.58 · 10 ³	a* c 1.21 · 10 ⁵ ± 1.10 · 10 ⁴	a* d			← 9.24 · 10 ³
2.2 Treated with ozonated water for 10 min	2.98 · 10 ² ± 3.36 · 10 ¹	a* a 6.50 · 10 ⁴ ± 1.96 · 10 ³	b* b 1.03 · 10 ⁵ ± 6.22 · 10 ³	a* c 2.37 · 10 ⁵ ± 1.30 · 10 ⁴	a* d			← 1.03 · 10 ⁴
3.2 Treated with ozonated water for 15 min	1.68 · 10 ² ± 3.49 · 10 ¹	a* a 7.58 · 10 ⁴ ± 2.36 · 10 ³	cb* b 1.21 · 10 ⁵ ± 5.12 · 10 ³	a* c 1.34 · 10 ⁵ ± 6.55 · 10 ³	a* d			← 6.14 · 10 ³
4.2 Treated with ozonated water for 20 min	2.73 · 10 ² ± 4.45 · 10 ¹	a* a 5.53 · 10 ⁴ ± 2.25 · 10 ³	db* b 1.53 · 10 ⁵ ± 8.66 · 10 ³	a* c 1.76 · 10 ⁵ ± 9.64 · 10 ³	a* d			← 9.33 · 10 ³
5.2 Treated with tap water	3.98 · 10 ³ ± 3.49 · 10 ²	b* a 3.55 · 10 ⁵ ± 1.39 · 10 ⁴	e* b 4.23 · 10 ⁵ ± 1.97 · 10 ⁴	b* c 6.71 · 10 ⁵ ± 2.07 · 10 ⁴	a* d			← 1.89 · 10 ⁴
6.2 Control carrots	5.23 · 10 ⁵ ± 1.96 · 10 ⁴	c a 5.57 · 10 ⁵ ± 2.56 · 10 ⁴	f b 1.85 · 10 ⁶ ± 1.30E + 05	c c 1.21 · 10 ⁸ ± 6.92 · 10 ⁶	b d			← 3.91 · 10 ⁶
<i>D</i> _{Dunnett}	3.39 · 10 ³ ↑	1.10 · 10 ⁴ ↑	4.97 · 10 ⁴ ↑	2.59 · 10 ⁶ ↑				

Population of moulds presented as the mean ± SD

The results are significant at the $P < 0.05$ level based on a one-way ANOVA with Dunnett's t -test ($n = 6$). Different superscripts letters in the same row (←)/column (↑) indicate significant differencesDifferences between control group and treatment groups (on different days) were analysed by using a one-way analysis of variance (ANOVA) with Dunnett's control post hoc tests
*Significant at the 0.05 level (in the column)

higher than on the control carrots. This growth was influenced by the higher storage temperature, which was higher than recommended. It was also observed that the outer peel of the carrot gradually withered. It is clear that the relative humidity, which at 16.0 ± 1.0 °C was half that at 3.00 ± 0.15 °C, had influenced the condition of the peel. However, the data (Table 1) showed that the total mould colony count on the carrots treated with ozonated water was lower. Moreover, after 36 days, there was a significant difference in the size of the mould populations between the carrots.

At the end of the entire storage period (day 36), the increase (1.50 log units) in the mould colony count of the carrots treated with ozonated water and stored at 3.00 ± 0.15 °C was 33.85% higher than the mould count on carrots treated with tap water (1.46 log units); however, it was 71.40% less than the control carrots (2.04 log units). By observing the mould populations through a light microscope, it could be seen that the treatment with ozonated water had inactivated the dominant moulds. However, over time, the moulds that were present as spores or were more resistant to ozone intensified their activity. When the total number of mould colonies on all the carrots were compared, the mould count on the carrots treated with ozonated water differed significantly from the counts on the other carrots. The total mould population count on the carrots treated with ozonated water was 92.66% (initially 94.05%) lower than the population count on the carrots treated with tap water and 99.99% (initially 99.96%) lower than the count on the control carrots.

The mould population count on the carrots treated with ozonated water at a storage temperature of 16.00 ± 1.00 °C was 75.14% (initially 94.05%) lower than

on the carrots treated with tap water and 99.86% (initially 99.95%) lower than on the control carrots at the same temperature.

The data in Fig. 2 shows that the population of moulds on carrots that were held at 3.00 ± 0.15 °C increased linearly. The regression coefficient is close to 1, indicating that the derived model fits the observed data.

More intense environmental penetration of mould was observed at 16.00 ± 1.00 °C than at 3.00 ± 0.15 °C. In treated carrots, the mould population increased to 1.95–2.65 logs units on day 8, increased by only 0.48–0.8 logs units from day 8 to day 20, and increased by 0.36–0.68 logs units from day 20 to day 36. At the beginning of storage, there was a tendency for the mould population to increase more at the higher storage temperature. After 8 days, the growth rate had stabilised and growth of mould population increased gradually with logarithmic trend.

The results of this study suggest that by treating carrots with ozonated water under appropriate conditions, the shelf life of the products can be extended for longer storage time (up to 1.70 times longer/24 days longer) than by treating with tap water. Naito and Takahara (2006) stated that a treatment with ozonated water increases the shelf life of vegetables, but that the total number of microorganisms does not significantly decrease.

Table 2 shows the intensity of the weight change from the carrots stored at 3.00 ± 0.15 °C and 16.00 ± 1.00 °C. On average, $2.33 \pm 0.10\%$ (23.33 ± 0.96 g kg⁻¹) of dirt was removed from the carrots ($n = 216$, $P < 0.05$). In Table 2, the Day 1 column shows the quantity of dirt that was removed by treating (amount of dirt from carrots “#”). Because the control carrots were not unrinsed, they are not shown in the day 1 column. The percent weight loss (with

Fig. 2 The characteristics of the mould growth population over time on carrots stored at 3.00 ± 0.15 °C

Table 2 Weight losses of the carrots at 3.00 ± 0.15 °C and at 16.00 ± 1.00 °C

Treatment	Weight losses, %					Regression results	
	Day 1	Day 8	Day 20	Day 28	Day 36	W, g	R ²
<i>3.00 ± 0.15 °C, Relative humidity—86.50 ± 2.00%</i>							
1.1 Treated with ozonated water for 5 min	3.08 ± 0.68 [#]	4.11 ± 0.58	10.98 ± 1.14	a* 14.21 ± 1.19	a* 17.45 ± 1.24	a 0.436τ + 1.865	0.97
2.1 Treated with ozonated water for 10 min	2.63 ± 1.27 [#]	4.32 ± 0.80	7.97 ± 0.92	b 10.92 ± 1.02	b* 13.87 ± 1.12	b* 0.323τ + 1.938	0.95
3.1 Treated with ozonated water for 15 min	2.99 ± 0.63 [#]	3.89 ± 0.58	8.71 ± 1.15	b 12.48 ± 0.83	c 16.24 ± 0.52	a 0.390τ + 1.600	0.96
4.1 Treated with ozonated water for 20 min	1.98 ± 0.59 [#]	3.68 ± 0.59	5.47 ± 0.65	cd* 9.16 ± 0.80	d* 12.85 ± 0.96	c* 0.262τ + 1.511	0.92
5.1 Treated with tap water	2.15 ± 0.84 [#]	2.46 ± 0.45	6.28 ± 0.72	cd* 9.40 ± 0.68	d* 12.52 ± 0.64	c* 0.963τ + 0.410	0.99
6.1 Control carrots	0	5.87 ± 0.51	10.76 ± 1.27	b 15.16 ± 1.14	ac 19.56 ± 1.00	a 0.535τ + 0.327	0.98
<i>D_{Dunnett}</i>							
<i>16.00 ± 1.00 °C, Relative humidity—41.60 ± 2.30%</i>							
1.2 Treated with ozonated water for 5 min	3.11 ± 0.66 [#]	6.33 ± 0.56	18.07 ± 0.90	a* 29.13 ± 0.97	a* 40.18 ± 1.04	a* 1.075τ − 0.637	0.97
2.2 Treated with ozonated water for 10 min	2.60 ± 1.28 [#]	5.23 ± 0.46	12.93 ± 0.48	b* 26.65 ± 0.70	b 40.38 ± 0.91	a* 1.067τ − 2.298	0.93
3.2 Treated with ozonated water for 15 min	2.96 ± 0.69 [#]	6.12 ± 0.58	16.61 ± 0.86	c 26.48 ± 0.95	b 36.36 ± 1.03	b 0.966τ − 0.270	0.98
4.2 Treated with ozonated water for 20 min	2.64 ± 0.67 [#]	6.39 ± 0.52	18.63 ± 0.72	a* 27.63 ± 0.74	b 36.64 ± 0.76	b 0.993τ − 0.073	0.99
5.2 Treated with tap water	2.86 ± 0.79 [#]	7.20 ± 0.44	18.10 ± 0.79	a* 27.17 ± 0.71	b 36.25 ± 0.63	b 0.271τ + 1.381	0.93
6.2 Control carrots	0	8.12 ± 0.55	19.08 ± 0.70	c 29.23 ± 0.91	b 39.39 ± 1.13	b 1.105τ − 1.386	0.99
<i>D_{Dunnett}</i>							
Population of moulds presented as the mean ± SD							
The results are significant at the $P < 0.05$ level based on a one-way ANOVA with Dunnett's t -test ($n = 6$). Different superscripts in the same row column (†) indicate significant differences							
Differences between control group and treatment groups (on different days) were analysed by using a one-way analysis of variance (ANOVA) with Dunnett's control post hoc tests							
*Significant at the 0.05 level (in the column)							
† Amount of dirt from carrots ($n = 36$, $P < 0.05$)							
## The average amount of dirt that was washed from the carrots, which was up to $2.33 \pm 0.10\%$ ($n = 216$, $P < 0.05$), was subtracted from the average weight loss							

the exception of samples 6.1 and 6.2) are shown in Table 2 (without dirt) in the day 8, 20, 28, and 36 columns. These data indicate that the weight loss of the carrots proportionally increased at both temperatures for carrots treated with tap water or ozonated water and for control carrots.

The weight loss of the control carrots included the loss of residual dirt; during storage, the dirt dried and some dirt was lost. In assessing the average weight loss of carrots after 36 days, the amount of dirt on the control carrots was also assessed (the data in Table 2 are set to “##” after the subtraction of the dirt index).

The carrots weight during the storage period (36 days) decreased linearly at temperatures of 3.00 ± 0.15 °C and 16.00 ± 1.00 °C. The regression equations for carrot weight loss are presented in Table 2. The R^2 values were high in all cases, varying from 0.916 to 0.989. Therefore, the derived regression model was appropriate for the observed data.

At 3.00 ± 0.15 °C, the carrots with the lowest weight losses were the carrots treated with tap water (125.20 ± 6.38 g kg⁻¹) and ozonated water for 20 min (128.50 ± 9.63 g kg⁻¹). The carrots with the largest weight losses were the carrots treated with ozonated water for 5 min (174.52 ± 12.35 g kg⁻¹) and the control carrots (172.33 ± 10.02 g kg⁻¹). On all days, except on day 8, there was a significant difference between the carrots from the control group and the carrots treated with tap water and ozonated water for 20 min (Dunnett post hoc tests). There was also a significant difference between days 20 and 28 for carrots treated with ozone (5 and 10 min) and between days 28 and 36 for the control group. No significant differences between the control group and carrots treated with ozonated water for 15 min. At 16.00 ± 1.00 °C, the largest weight losses were seen in the carrots that were treated with ozonated water for 10 min (401.85 ± 10.37 g kg⁻¹) and 5 min (403.79 ± 9.15 g kg⁻¹). The largest difference was observed between the control group and the carrots that were treated with ozonated water for 5 min.

A comparison of the weight loss of all the carrot groups (control and treated) that were stored at 3.00 ± 0.15 °C shows that treating with ozonated water had a minimal effect on the outer peel of the carrot. A more distinct difference was observed at 16.00 ± 1.00 °C. Among the treatments with ozonated water, the use of longer treatments resulted in less variable weights. Among the different groups of carrots treated with ozonated water, the weight variation was lower in groups with longer treatment. However, the carrots with the largest weight loss had more sprouted leaves. It has been suggested that ozone activates germination, and this increases the rate of respiration. Ali et al. (2014) reports that the largest weight loss occurs through stem scar, stomata and the cuticle. Consequently, the amount of water lost may vary depending on

the thickness of the cuticle, which varies with the species and degree of maturation.

Fresh product weight loss depends on the type of the product. The changes in fresh carrot weight relative to the ozone concentration used for treating has been examined in several studies. Other studies with ozone water contradict and report that ozone had no significant effect on carrot weight and carrot strength. Hildebrand et al. (2008) and Liew and Prange (1994) conducted studies that showed that the treatment of carrots with ozone concentrations of 50.00 ± 10.00 nL L⁻¹ and 7.50 – 60.00 µL L⁻¹ had no effect on carrot weight. In a study conducted by Souza et al. (2018), treatment with ozonated water up to 10.00 mg L⁻¹ had no effect on weight loss up to 5 days of storage. That study also suggested that there is a limit to ozone concentrations that must be determined for each product and cannot be exceeded, because excess ozone can damage the product (Souza et al. 2018). When comparing the ozone usage in other vegetable treatments it should be noted that few studies had no significant weight loss, because relatively low concentrations were used. For example, after processing tomatoes with 20.00 µg L⁻¹ ozone for 10 min (Rodoni et al. 2009) and paprika with 2.00 µg L⁻¹ of ozone for 1–5 min (Horvitz and Cantalejo 2012), there was no significant weight loss.

At the end of the research (36 days, Table 2), the carrots treated with ozonated water and then stored at 3.00 ± 0.15 °C had lost 174.52 ± 12.35 g kg⁻¹ of their weight. Evaporation from the carrots stored at 16.00 ± 1.00 °C was more intense because the relative humidity ($41.60 \pm 2.30\%$) and high temperature were unfavourable for storage, and the carrots withered faster. At 16.00 ± 1.00 °C, the carrots lost 401.80 ± 10.37 g kg⁻¹ of their weight, and the losses were 56.57% higher than in carrots stored at 3.00 ± 0.15 °C.

In the case of using the recommended storage conditions to type for a particular product, it is possible to prevent or reduce weight loss.

According to Ölmez and Akbas (2009), the most important goal of processing technology is to ensure the safety and quality of fresh vegetables by extending the shelf life and ensuring disinfection. With regard to safe storage and quality, products treated with ozonated water can be kept up to 1.70 times longer. Moreover, chemicals such as chlorine that commonly enter sewage systems or the environment are not required. Karaca and Velioglu (2007) reported that chlorine can produce trihalomethanes, which create concerns for both human food safety and the environment. Although low concentrations of chemicals are used in the water for rinsing products, they pose risks to human or animal health because these chemicals are stable and accumulate in the food chain.

The cycles of oxygen and ozone are similar in nature to the cycle of water. Although ozone is an effective disinfectant, its half-life is very short (Tiwarei et al. 2010). During its short lifetime, ozone is capable of destroying up to 99.00% of microorganisms such as bacteria, viruses and other. The reduced ozone then reverts to the oxygen state. Ozone disintegration does not leave any by-products, and the water used for treating a product can be used again. Water treated with ozone is clean and can be used for subsequent treating by replacing the volume of water that is removed with the product. Therefore, the additional water demand is low.

The production of ozone requires extra energy; however, the quality and shelf life of the product are maintained for a longer period of time. Miller et al. (2013) and Horvitz and Cantalejo (2014) suggest that ozone technologies are economically advantageous. The extra energy that is required for carrot treatment is compensated by higher quality of products with longer shelf life.

By combining the recommended storage conditions for the plant product and product treatment with ozone, products can be stored for longer time periods (up to 1.70 times longer). By using ozone, the population of microorganisms can be reduced, the safe storage of a product can be prolonged, and the storage costs of the product can be reduced.

Conclusion

Mould colonies were inactivated within the first 5 min of treatment of carrots with ozonated water at an ozone concentration of $1.53 \pm 0.09 \text{ mg L}^{-1}$. The treatment successfully reduced mould colonies by up to 99.99%. Storage can be prolonged by an average of 1.7 times (24 days longer) by treating carrots with ozonated water at an ozone concentration of $1.53 \pm 0.09 \text{ mg L}^{-1}$ for 5 min during preparation and by storing vegetables under appropriate conditions (at $3.00 \pm 0.15 \text{ }^{\circ}\text{C}$) in comparison to carrots treated under tap water. Additionally, the treatment of carrots with ozonated water had only minimal effect on their weight. Additional research will include testing ozonated water spray technology and will attempt to determine ozone's effectiveness in preventing the formation of mould colonies.

References

- Ali A, Ong MK, Forney CF (2014) Effect of ozone pre-conditioning on quality and antioxidant capacity of papaya fruit during ambient storage. *Food Chem* 142:19–26. <https://doi.org/10.1016/j.foodchem.2013.07.039>
- Baba S, Satoh S, Yamabe C (2002) Development of measurement equipment of half life of ozone. *Vacuum* 65:489–495. [https://doi.org/10.1016/S0042-207X\(01\)00461-4](https://doi.org/10.1016/S0042-207X(01)00461-4)
- Carletti L, Botondi R, Moschetti R, Stella E, Monarca D, Cecchini M, Massantini R (2013) Use of ozone in sanitation and storage of fresh fruits and vegetables. *Food Agric Environ* 11:585–589
- Chen Z, Guo X, Wu T (2016) A novel dehydration technique for carrot slices implementing ultrasound and vacuum drying methods. *Ultrason Sonochem*. <https://doi.org/10.1016/j.ultsonch.2015.11.026>
- da Silva MV, Gibbs PA, Kirby RM (1998) Sensorial and microbial effects of gaseous ozone on fresh scad (*Trachurus trachurus*). *J Appl Microbiol* 84:802–810
- de Alencar ER, Faroni LRD, Martins MA, de Costa AR, Cecon PR (2011) Decomposition kinetics of gaseous ozone in peanuts. *Eng Agric* 31:930–939. <https://doi.org/10.1590/S0100-69162011000500011>
- Ekici L, Ozturk I, Karaman S, Caliskan O, Tornuk F, Sagdic O, Yetim H (2015) Effects of black carrot concentrate on some physicochemical, textural, bioactive, aroma and sensory properties of sucuk, a traditional Turkish dry-fermented sausage. *LWT Food Sci Technol* 62:718–726. <https://doi.org/10.1016/j.lwt.2014.12.025>
- Fernández-Cruz ML, Mansilla ML, Tadeo JL (2010) Mycotoxins in fruits and their processed products: analysis, occurrence and health implications. *J Adv Res* 1:113–122. <https://doi.org/10.1016/j.jare.2010.03.002>
- Fishburn JD, Tang Y, Frank JF (2012) Efficacy of various consumer-friendly produce washing technologies in reducing pathogens on fresh produce. *Food Prot Trends* 32:456–466
- Gonçalves AA (2009) Ozone—an emerging technology for the seafood industry. *Braz Arch Biol Technol* 52:1527–1539. <https://doi.org/10.1590/S1516-89132009000600025>
- Hartikainen H, Svanes E, Franke U, Mogensen L, Andersson S, Bond R, Burman C, Einarsson E, Eklöf P, Joensuu K, Olsson ME, Rääkkönen R, Sinkko T, Stubhaug E, Rosell A, Sundin S (2017) Food losses and waste in primary production—case studies on carrots, onions, peas, cereals and farmed fish. Nordic Council of Ministers, Denmark
- Hildebrand PD, Forney CF, Song J, Fan L, McRae KB (2008) Effect of a continuous low ozone exposure (50 nLL^{-1}) on decay and quality of stored carrots. *Postharvest Biol Technol* 49:397–402. <https://doi.org/10.1016/j.postharvbio.2008.03.012>
- Horvitz S, Cantalejo MJ (2012) Effects of ozone and chlorine postharvest treatments on quality of fresh-cut red bell peppers. *Int J Food Sci Technol* 47:1935–1943. <https://doi.org/10.1111/j.1365-2621.2012.03053.x>
- Horvitz S, Cantalejo MJ (2014) Application of ozone for the postharvest treatment of fruits and vegetables. *Crit Rev Food Sci Nutr* 54:312–339. <https://doi.org/10.1080/10408398.2011.584353>
- Huyskens-Keil S, Hassenberg K, Herppich WB (2011) Impact of postharvest UV-C and ozone treatment on textural properties of white asparagus (*Asparagus officinalis* L.). *J Appl Bot Food Qual* 84:229–234
- Karaca H, Velioğlu YS (2007) Ozone applications in fruit and vegetable processing. *Food Rev Int* 23:91–106. <https://doi.org/10.1080/87559120600998221>
- Khadre MA, Yousef AE, Kim J-G (2006) Microbiological aspects of ozone applications in food: a review. *J Food Sci* 66:1242–1252. <https://doi.org/10.1111/j.1365-2621.2001.tb15196.x>
- Liew CL, Prange RK (1994) Effect of ozone and storage temperature on postharvest diseases and physiology of carrots (*Daucus carota* L.). *J Am Soc Hortic Sci* 119:563–567

- Miller FA, Silva CLM, Brandão TRS (2013) A review on ozone-based treatments for fruit and vegetables preservation. *Food Eng Rev* 5:77–106. <https://doi.org/10.1007/s12393-013-9064-5>
- Naito S, Takahara H (2006) Ozone contribution in food industry in Japan. *Ozone Sci Eng* 28:425–429. <https://doi.org/10.1080/01919510600987347>
- Ölmez H, Akbas MY (2009) Optimization of ozone treatment of fresh-cut green leaf lettuce. *J Food Eng* 90:487–494. <https://doi.org/10.1016/j.jfoodeng.2008.07.026>
- Pandey H, Kumar V, Roy BK (2014) Assessment of genotoxicity of some common food preservatives using *Allium cepa* L. as a test plant. *Toxicol Rep* 1:300–308. <https://doi.org/10.1016/j.toxrep.2014.06.002>
- Pascual A, Llorca I, Canut A (2007) Use of ozone in food industries for reducing the environmental impact of cleaning and disinfection activities. *Trends Food Sci Technol* 18(Supplement 1):S29–S35. <https://doi.org/10.1016/j.tifs.2006.10.006>
- Pavia M, Pileggi C, Nobile CGA, Angelillo IF (2006) Association between fruit and vegetable consumption and oral cancer: a meta-analysis of observational studies. *Am J Clin Nutr* 83:1126–1134. <https://doi.org/10.1093/ajcn/83.5.1126>
- Pérez-Jiménez J, Saura-Calixto F (2015) Macromolecular antioxidants or non-extractable polyphenols in fruit and vegetables: intake in four European countries. *Food Res Int* 74:315–323. <https://doi.org/10.1016/j.foodres.2015.05.007>
- Pierron RJG, Pages M, Couderc C, Compant S, Jacques A, Violleau F (2015) In vitro and in planta fungicide properties of ozonated water against the esca-associated fungus *Phaeoacremonium aleophilum*. *Sci Hortic* 189:184–191. <https://doi.org/10.1016/j.scienta.2015.03.038>
- Pitt JJ, Hocking AD (2009) *Fungi and food spoilage*. Springer, New York
- Priyanka BS, Rastogi NK, Tiwari BK (2014) Opportunities and challenges in the application of ozone in food processing. In: Sun D (ed) *Emerging technologies for food processing*, 2nd edn. Academic Press, San Diego, pp 335–358
- Ramos B, Miller FA, Brandão TRS, Teixeira P, Silva CLM (2013) Fresh fruits and vegetables—an overview on applied methodologies to improve its quality and safety. *Innov Food Sci Emerg Technol* 20:1–15. <https://doi.org/10.1016/j.ifset.2013.07.002>
- Rodoni L, Casadei N, Concellón A, Chaves Alicia AR, Vicente AR (2009) Effect of short-term ozone treatments on tomato (*Solanum lycopersicum* L.) fruit quality and cell wall degradation. *J Agric Food Chem* 58:594–599. <https://doi.org/10.1021/jf9029145>
- Shankar R, Kaushik U, Bhat SA (2014) Food processing and preservation by ozonation. *Int J Sci Eng Technol* 02:354–370
- Smilanick JL (2003) Use of ozone in storage and packing facilities. In: Washington tree fruit postharvest conference. <http://postharvest.tfrec.wsu.edu/PC2003H.pdf>
- Souza LPd, Faroni LRD, Heleno FF, Cecon PR, Gonçalves TDC, Silva GJd, Prates LHF (2018) Effects of ozone treatment on postharvest carrot quality. *LWT Food Sci Technol* 90:53–60. <https://doi.org/10.1016/j.lwt.2017.11.057>
- Suslow TV (2004) Ozone applications for postharvest disinfections of edible horticultural crops. University of California: Division of Agriculture and Natural Resources Publication 8133:8. <https://anrcatalog.ucanr.edu/pdf/8133.pdf>
- Tiwari BK, Muthukumarappan K (2012) Ozone in fruit and vegetable processing. In: O'Donnell C, Tiwari BK, Cullen PJ, Rice RG (eds) *Ozone in food processing*, 1st edn. Blackwell Publishing Ltd., London, pp 55–80
- Tiwari BK, Brennan CS, Curran T, Gallagher E, Cullen PJ, O'Donnell CP (2010) Application of ozone in grain processing. *J Cereal Sci* 51:248–255. <https://doi.org/10.1016/j.jcs.2010.01.007>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.