

Effect of Ozone and Storage Temperature on Postharvest Diseases and Physiology of Carrots (*Daucus carota* L.)

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Abstract. Effects of ozone and storage temperature on carrots and two postharvest pathogens—*Botrytis cinerea* Pers. and *Sclerotinia sclerotiorum* de Bary—were investigated. Pathogen-inoculated and uninoculated whole carrots were exposed to an ozone concentration of 0 (control), 7.5, 15, 30, or 60 $\mu\text{l}\cdot\text{liter}^{-1}$. Treatment chambers were flushed with a total flow rate of 0.5 liters $\cdot\text{min}^{-1}$ (air and ozone) for 8 h daily for 28 days. The experiment was repeated twice at storage temperatures of 2, 8, and 16C. The residual ozone concentration (ozone supplied–exhausted and reacted ozone) increased with ozone supply concentration but was less at higher storage temperatures. A 50% reduction of daily growth rates of both fungi at the highest ozone concentration indicated that ozone was fungistatic. Carrot respiration rate, electrolyte leakage, and total color differences increased with ozone concentration. Ozone-treated carrots were lighter (higher L^* values) and less intense (lower chroma values) in color than control carrots.

Watery soft rot (*Sclerotinia sclerotiorum*) and gray mold (*Botrytis cinerea*) are common postharvest diseases of carrots. Until recently, growers used the fungicide benomyl to control these storage rots. The manufacturer's withdrawal of benomyl as a postharvest fungicidal dip on carrots has necessitated a search for alternatives; ozone is one potential candidate.

Ozone is the tri-atomic form of oxygen that is unstable and decomposes either spontaneously or after it comes in contact with oxidizable surfaces. Ozone's high oxidation potential (Lide, 1991) makes it an effective disinfectant for poultry hatcheries, poultry chill water, water treatment plants, soft-drink bottling plants, and aquaria water (Guinvarch, 1959; Rosenthal and Wilson, 1987; Sheldon and Brown, 1986; Torricelli, 1959; Whistler and Sheldon, 1989; Yang and Chen, 1979). Since ozone can be easily and economically generated on site, transportation and storage costs are not incurred. Unlike CO₂ and N, which are two gases commonly used in storage, ozone has a characteristic odor. For this reason, harmful levels of ozone can be instantly detected and avoided by workers.

Ozone can be applied as a gas or as ozonated water. Reported differences on the effectiveness of ozone as a storage disinfectant may be due to differing application methods or measurements of ozone concentration, treatment period, and pathogen and product sensitivity to ozone. Gibson et al. (1960) and Rice et al. (1983) concluded that ozone is an effective agent for controlling microbial and fungal pathogens in stored produce such as cheese, strawberries, raspberries, currants, bananas, and potatoes. Continuous exposure to 0.05 ppm ozone (gas) effectively killed *Escherichia coli* after 3 days and *Staphylococcus aureus* in vitro after 15 days (Kashiwagi et al., 1987). Ogawa et al. (1990) demonstrated that spores of *B. cinerea* on the surface of tomatoes were inactivated by exposure to 3.8 g of ozone/ml of water in 10 min. Other reports,

however, have suggested that ozone is ineffective in controlling rots on apples, muskmelons, peaches, strawberries, blueberries, and green beans. Ozonated apples, cantaloupes, cranberries, corn kernels, and cereal grains demonstrated more decay or damage than those not ozonated (Barger et al., 1948; Brooks and Csallany, 1978; Naito, 1989; Norton, 1968; Schomer and McCulloch, 1948; Spalding, 1968). The objectives of this study were 1) to determine the residual concentration of ozone, 2) to determine the effect of ozone on the two major storage pathogens of carrots, and 3) to observe ozone-induced changes in carrot physiology and quality during storage.

Methods and Materials

'Vitabrite' carrots, obtained from a local grower (Berwick, N.S.), were hand-washed and stored at 0C until use. Crown diameters of the carrots were from 3 to 4 cm.

An ozone generator (Tri-Ox, Swindon, England) was set to produce 76.5 $\mu\text{l}\cdot\text{liter}^{-1}$ of ozone in air. Air containing ozone at flow rates of 0 (control), 0.05, 0.1, 0.2, or 0.4 liters $\cdot\text{min}^{-1}$ were blended with compressed air to produce ozone concentrations of 0, 7.5, 15, 30, or 60 $\mu\text{l}\cdot\text{liter}^{-1}$, in a total flow of 0.5 liters $\cdot\text{min}^{-1}$ for each treatment. Ozone and compressed air flows were controlled with needle valves (Nupro Co., Willoughby, Ohio). Treatment chambers consisted of air-tight 64-liter polyvinylchloride containers (IPL, St. Damien, Que.) placed in storage rooms set at 2, 8, or 16C. The chambers were flushed continuously for 8 h daily for 28 days.

Ozone concentrations in the chambers were monitored during the treatment period with an ultraviolet-based detector with a measurement range of 0 to 100 $\mu\text{l}\cdot\text{liter}^{-1}$ at 253.7 nm (ozone analyzer model IN-2000-5; In-USA, Newtonville, Mass.). Values presented in Table 1 represent stable concentrations after 4 h of treatment.

Disease. Isolates of *S. sclerotiorum* and *B. cinerea* were obtained from infected carrots in local storage. Fungal stock cultures and inoculum were maintained on potato dextrose agar (PDA) (Becton-Dickinson, Cockeysville, Md.).

A 1.0-cm-diameter mycelial plug, obtained near the margin of a 4- to 5-day-old fungal culture, was placed in a wound of each carrot 1.0 \pm 0.5 cm from the crown. The wound was a 1.0-cm-diameter \times 0.5-cm-deep depression created with a 1.0-cm-diam-

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eter corkborer. Fungal growth along the length of the carrot was measured once every 7 days for 28 days. A sample of 10 carrots was inoculated separately with each fungus for the 15 combinations of ozone concentration and temperature. The entire experiment was repeated twice.

Physiology. Fifteen carrots from each treatment were monitored for weight loss and color changes over the 28-day test period (initial and final measurements were recorded). Carrot moisture loss was measured as percentage weight loss [(initial weight – final weight)/initial weight × 100]. Initial and final carrot color readings, using Commission International d’Eclairage (CIE) L*, a*, b* color space coordinates, were obtained with a tristimulus colorimeter. The Chroma Meter CR-200 (Minolta, Ramsey, N.J.) has an 8-mm-diameter measuring port that uses diffuse illumination and a 0° viewing angle. The meter was calibrated with a white standard calibration plate. Colorimetric values for hue (color) and chroma (intensity) were calculated as chroma = $\sqrt{a^2 + b^2}$ and hue = $\cos^{-1} [a/\sqrt{a^2 + b^2}]$ (Little, 1975). Total color difference was calculated as $(\Delta E) = \sqrt{[(L_{fin} - L_{ini})^2 + (a_{fin} - a_{ini})^2 + (b_{fin} - b_{ini})^2]}$ (Gnanasekharan et al., 1992). Color measurements were taken between 1 to 2 cm from the carrot crown for initial and final readings.

After 28 days, five preweighed carrots were selected from each ozone treatment for respiration measurements at 9C. The carrots were placed in Nalgene jars and continuously flushed with 25 to 35 ml·min⁻¹ of CO₂-free air. Carbon dioxide evolution rates were measured daily for 5 days with a gas chromatograph (model 3400; Varian Instruments, Mississauga, Ont.) equipped with a CRT-1 column (Alltech Assn., Guelph, Ont.) and a thermal conductivity detector.

A conductivity meter (Radiometer Conductivity Meter, Copenhagen) was used to measure electrolyte leakage of 5 carrots after the experimental period. Initial electrolyte leakage was determined after a 24-h incubation of 1 g of carrot peel from each carrot in 20 ml of distilled water at 5C. The water contained 1 µl·liter⁻¹ Tween 20 to reduce surface tension (Prange and Lidster, 1991). The peels and bathing solution were then frozen at –18C and held for another 24 h before total conductivity was measured on the thawed solution. Percent total solute leakage was calculated as initial conductivity/final conductivity × 100 (Beckerson and Hofstra, 1980).

The two factors, temperature and ozone concentration, were arranged in a split-plot design and replicated twice. Temperatures were assigned to the storage rooms (main plot), and the ozone concentrations (split plot) were randomized to chambers within each storage room. The results were analyzed using the analysis of variance directive in Genstat 5 (Payne et al., 1988).

A logarithmic (log₁₀) transformation was used to stabilize variance of fungal growth rates. Mean log values were back-transformed and are presented in parentheses in Table 2.

Results

Ozone. The quantitative factor of ozone concentration can be expressed as either supplied or residual ozone concentration. Supplied ozone concentration refers to the ozone concentration provided to the chambers, whereas residual concentration was the ozone concentration measured in a chamber containing experimental carrots (residual concentration = supplied concentration – exhausted and reacted ozone) (Table 1).

In this study, residual ozone concentration seemed to be influenced by temperature and supplied ozone concentration (Table 1). It seems that the rate of ozone depletion increased with temperature since higher temperatures produced lower residual ozone concen-

trations.

To help identify the influence of ozone concentration at each of the temperatures, regression coefficients of the variate means at the three temperatures have been provided in Tables 2–4. Significant differences in regression coefficients among the treatment temperatures would indicate a different linear effect of ozone concentration for each temperature.

Disease. The fungal inoculum was applied to wounded carrots to ensure disease development. Fungal surface growth included spores, sclerotia and mycelia of *B. cinerea*, and only sclerotia, and mycelia of *S. sclerotiorum*. Both fungi developed in the ozonated environment but exhibited varied mycelial growth rates and surface structure (sclerotia and spores) development on the carrots. At 2C there were fewer surface structures present with increasing ozone concentration (data not shown). At the highest ozone concentration (60 µl·liter⁻¹), water-soaked lesions (cellular necrosis) were the only indicators of fungal growth; however, mycelia developed on the carrots after removal from the ozonated atmospheres.

Growth rates of *B. cinerea* and *S. sclerotiorum* increased with temperature, with most of the increase occurring between 2 and 8C (Table 2). A trend of decreased growth rate with increased ozone concentration was observed within each storage temperature. The growth rate for *B. cinerea* at 2, 8, and 16C at 60 µl·liter⁻¹ ozone was 43% to 58% of the respective control treatment.

Although growth rate of *S. sclerotiorum* increased with storage temperature, the magnitude of the increase was reduced with higher ozone concentration (Table 2). The growth rate of *S. sclerotiorum* at 2, 8, and 16C was reduced to between 44% to 63% at 60 µl·liter⁻¹ compared with the respective control treatments. The lower linear and quadratic regression coefficients at 2C for *S. sclerotiorum* than at 8 and 16C suggests that the higher residual ozone concentration at 2C (Table 1) could have caused a greater reduction in fungal growth rate than those observed at 8 and 16C. This effect was not observed for *B. cinerea*.

Physiology. Ozone did not affect carrot weight loss (data not presented). Carrot respiration rate at 9C generally increased with ozone concentration, with a mean ranging from 18.2 mg CO₂/kg per h in the control to 20.7 mg CO₂/kg per h at 60 µl·liter⁻¹ ozone (Table 3). Even though the respiration rate was higher in carrots treated with ozone, carrot leaves, which appeared at 8 and 16C, were fewer, shorter, and necrotic in the treatments receiving ozone (data not shown). The presence of carrot leaves decreased with increasing ozone concentration.

Ozone treatment increased electrolyte leakage from 37.4% in the control to 48.8% at 60 µl·liter⁻¹ ozone. The highest electrolyte leakages occurred at 2 and 8C. The linear regression coefficient at 16C was lower than at 2 and 8C and could be caused by the existence of higher residual ozone concentrations at the two lower temperatures (Table 1).

Visual observations of carrots agreed with the results of electrolyte leakage. Carrot surfaces in ozone treatments were pitted with dry white blotches, which intensified with increas-

Table 1. The effect of temperature and ozone supply on residual concentration.

Temp (°C)	Ozone supply (µl·liter ⁻¹)				
	0	7.5	15	30	60
2	0	1.0	3.1	10	22
8	0	1.0	3.0	6.7	20
16	0	0.6	1.8	3.0	18

Table 2. Daily growth rate of *Botrytis cinerea* and *Sclerotinia sclerotiorum* on carrots at different temperatures and ozone concentrations in storage.

Storage temp (°C)	Ozone concn (µl-liter ⁻¹)					Regression coefficient (×10 ⁻³)		
	0	7.5	15	30	60	Linear	Quadratic ^z	
<i>Botrytis cinerea</i> (log ₁₀ mm-day ⁻¹)								
2	-0.302 (0.499) ^y	-0.396 (0.401)	-0.459 (0.347)	-0.629 (0.237)	-0.667 (0.215)	-6.0	0.14	
8	0.306 (2.02)	0.250 (1.78)	0.130 (1.35)	0.090 (1.23)	0.071 (1.18)	-6.3	0.12	
16	0.379 (2.39)	0.323 (2.10)	0.117 (1.31)	0.044 (1.11)	0.036 (1.09)	-6.8	0.17	
SEM ^x (n = 20, df = 12): 0.0417					SE (n = 2, df = 3):		0.620	0.037
Significant effects (P < 0.05): T, O _{l,q} ^w								
<i>Sclerotinia sclerotiorum</i> (log ₁₀ mm-day ⁻¹)								
2	-0.275 (0.531)	0.331 (0.466)	-0.395 (0.403)	-0.498 (0.318)	-0.631 (0.234)	-5.9	0.05	
8	0.472 (2.96)	0.389 (2.49)	0.315 (2.07)	0.345 (2.21)	0.271 (1.87)	-2.7	0.06	
16	0.607 (4.05)	0.514 (3.27)	0.487 (3.06)	0.406 (2.55)	0.382 (2.41)	-3.4	0.10	
SEM (n = 20, df = 12): 0.0497					SE (n = 2, df = 3):		0.65	0.038
Significant effects (P < 0.05): T, O _{l,q} , T × O _l								

^zOrthogonalized coefficient.

^yBack-transformed mean (mm-day⁻¹).

^xTo compare effect of temperature within ozone supply concentration.

^wT = temperature, O_l = linear effect for ozone, O_q = quadratic effect for ozone.

Table 3. Respiration rate and electrolyte leakage of carrots stored for 28 days at different temperatures and ozone concentrations.

Storage temp (°C)	Ozone supply concn (µl-liter ⁻¹)					Regression coefficient (×10 ⁻²)	
	0	7.5	15	30	60	Linear	
<i>Respiration rate</i> (mg CO ₂ /kg per h) ^z							
2	11.4	11.4	13.4	16.8	15.3	0.75	
8	19.7	17.3	18.2	17.8	22.8	0.65	
16	23.4	23.5	20.4	21.6	24.2	0.16	
SEM ^y (n = 2, df = 12): 2.21					SE (n = 2, df = 3):		0.344
Significant effects (P < 0.05): O _l ^w							
<i>Electrolyte leakage</i> (%)							
2	39.3	44.2	41.3	43.2	53.2	20.8	
8	36.3	42.6	41.4	43.6	53.1	24.6	
16	36.6	36.9	36.3	40.8	40.0	7.1	
SEM (n = 10, df = 12): 3.38					SE (n = 2, df = 3):		2.7
Significant effects (P < 0.05): O _l , T × O _l							

^zRespiration rate is the mean of five daily measurements at 9C.

^yTo compare effect of temperature within ozone supply concentration.

^wT = temperature, O_l = linear effect for ozone.

ing ozone concentration. Visible injury in the form of brown water-soaked lesions also appeared on carrot leaves treated with the highest ozone concentration at 2C.

The normal orange-red surface color of carrots appeared to be bleached on ozone-treated carrots. This was reflected in mean L* values, which increased from 49.6 in the control to 52 at an ozone concentration of 60 µl-liter⁻¹ (Table 4). Ozone also affected the intensity of carrot color. Mean chroma values of 30.2 in the control decreased to 28.7 at 60 µl-liter⁻¹ ozone (Table 4). Although not significant, the decrease in mean chroma was greatest at 2C, followed by 8 and 16C. Although hue was unaffected by the ozone treatments, higher hue values were obtained at a storage temperature of 8C (Table 4). Mean total color differences in the control

increased from 4.2 to 6.6 when treated with an ozone concentration of 60 µl-liter⁻¹ (Table 4).

Discussion

Although some storage studies have reported ozone concentrations as residual ozone (Schomer and McCulloch, 1948; Spalding, 1968), experiments using residual ozone concentration may not be appropriate since residual ozone concentration may be affected by temperature and reactivity of exposed materials. Ewell (1933) reported that applying 1 ppm ozone to produce such as eggs, apples, and beef (with similar amounts of surface area) resulted in different residual ozone concentrations. This study indicated that

Table 4. Lightness, chroma, hue and total color difference of carrots stored for 28 days at different temperatures and ozone concentrations.

Storage temp (°C)	Ozone concn ($\mu\text{l}\cdot\text{liter}^{-1}$)					Regression coefficient ($\times 10^{-2}$)		
	0	7.5	15	30	60	Linear	Quadratic ^z	
<i>Lightness (L*)</i>								
2	49.5	50.2	51.2	51.4	51.5	2.9	-0.17	
8	49.3	49.8	50.4	51.2	52.1	4.6	-0.059	
16	49.9	50.7	50.9	51.2	52.3	3.5	-0.019	
SEM ^y (n = 30, df = 12): 0.558					SE (n = 2, df = 3)		0.686	-0.0408
Significant effects ($P < 0.05$): $O_{1,q}^x$								
<i>Chroma</i>								
2	30.2	29.4	28.4	27.1	26.6	-5.8		
8	29.7	31.1	31.4	30.3	28.8	-2.8		
16	30.6	31.0	30.9	30.1	30.6	-0.6		
SEM (n = 30, df = 12): 1.21					SE (n = 2, df=3):		1.46	
Significant effects ($P < 0.05$): O_1								
<i>Hue</i>								
2	49.0	47.2	47.6	46.7	47.1	-2.2		
8	49.1	50.6	48.8	49.1	48.8	-1.4		
16	48.3	48.3	49.3	48.8	48.2	-0.29		
SEM (n = 30, df = 12): 0.694					SE (n = 2, df = 3):		1.58	
Significant effects ($P < 0.05$): T ^x								
<i>Total color difference</i>								
2	3.3	3.9	3.8	4.3	5.8	3.4		
8	4.4	2.8	3.9	3.8	7.3	5.8		
16	4.9	3.5	4.9	4.9	6.6	3.7		
SEM (n = 30, df = 12): 1.07					SE (n = 2, df = 3):		1.94	
Significant effects ($P < 0.05$): O_1								

^zOrthogonalized coefficient.

^yTo compare effect of temperature within ozone supply concentration.

^xT = temperature, O_1 = linear effect for ozone, O_q = quadratic effect for ozone.

the residual concentration was influenced by temperature. Increasing the storage temperature would increase the amount of ozone required to maintain a specific residual concentration. Therefore, maintaining a constant residual ozone concentration over a storage period will require adjustments in the amount of ozone supplied. These adjustments could be made with an automated gas control system to maintain gas concentrations, such as the ozone application and measurement systems frequently used by environmental and pollution researchers (Hale-Marie et al., 1991).

Our results suggest that the efficacy of ozone as a disinfectant must be individually assessed for each commodity at its ideal storage temperature. Furthermore, the quantity of produce in storage and the system available for dispensing and measuring ozone must be considered.

The effect of ozone on *B. cinerea* and *S. sclerotiorum* was fungistatic and not fungicidal. Although some inhibitory effects were observed with ozone residual concentrations of 10 to 22 $\mu\text{l}\cdot\text{liter}^{-1}$ at 2C, these concentrations seemed to cause physical and physiological damage to carrots. Symptoms of physiological disruptions included increased respiration rates, electrolyte leakage, and color changes. Higher respiration rates with increasing ozone concentrations are likely an expression of abnormal metabolism or injury caused by ozone to the carrots. Further, alterations in appearance of ozone-treated carrots due to color changes and surface pitting may affect consumer appeal.

The results of this study agree with other studies that have examined the effects of ozone on plants (Beckerson and Hofstra, 1980; Frederick and Heath, 1975; Hewitt et al., 1990; Sakaki et al., 1983; Tomlinson and Rich, 1970). These studies demonstrated

that plants exposed to ozone have higher electrolyte leakage compared with control plants (no ozone) and are subjected to pigment destruction (chlorophyll a and carotenoids). Some researchers have speculated that ozone causes some form of lipid peroxidation in the plant cells, a result suggesting that membrane lipids are susceptible to ozone damage. In addition, physiological disruptions to plant tissue caused by ozone treatment may also result in losses of organic and inorganic nutrients.

If ozone is to be used for carrot storage, a balance must be found between preserving carrot quality and effective disease control. This study has demonstrated that reducing temperature from 16 to 2C significantly reduced fungal growth of nonozonated carrots. Immediate ozonation may be unnecessary if carrots are properly harvested, washed, hydro-cooled, and cold-stored at the appropriate temperature. Although commercial carrot storages have air temperatures between 0 and 1C, carrots stored in large bins may build up pockets of heat in the middle of the bins during long storage periods. These heat pockets are prime areas for fungal growth and development. It is often the combination of reduced resistance to infection by the carrots and poor heat removal that encourages pathogen proliferation. With proper air circulation and temperature control, ozonation may be introduced in the latter part of the storage period, when carrots are less resistant to fungal attacks (Goodliffe and Heale, 1978; Harding and Heale, 1980). Even at low temperatures, diseases can spread by mycelial growth and spore production. Since carrot diseases often spread from one root to the next from a focus of infection or from contaminated storage bins, reducing surface fungal growth with ozone

may limit the spread of pathogens. Based on our results, an ozone supply of 15 $\mu\text{l-liter}^{-1}$ for 8 h a day at 2C could provide some disease protection with a minimum of physical and physiological damage.

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