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ARTICLE

Effect of Short-Term Ozone Treatments on Tomato (*Solanum lycopersicum* L.) Fruit Quality and Cell Wall Degradation

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We evaluated the effect of short-term gaseous ozone treatment (10 μL/L; 10 min) on tomato fruit quality and cell wall degradation. The treatments did not modify fruit color, sugar content, acidity, or antioxidant capacity but reduced fruit damage and weight loss and induced the accumulation of phenolic compounds. In addition, softening was delayed in ozone-treated fruit. Cell wall analysis showed that exposure to ozone decreased pectin but not hemicellulose solubilization. Polyuronide depolymerization was also reduced in ozone-treated fruit. While the treatments did not alter the activity of the pectin-degrading enzymes polygalacturonase (PG) and β-Galactosidase (β-Gal), a clear decrease in pectin methyl esterase (PME) was found. Results show that short-term ozone treatments might be useful to reduce fruit damage and excessive softening, two of the main factors limiting tomato postharvest life, without negatively affecting other quality attributes. The impact of the treatments on fruit softening might be associated with reduced disassembly (solubilization and depolymerization) of pectic polysaccharides.

KEYWORDS: Pectin; hemicellulose; cell wall degrading enzymes; postharvest; softening; storage

INTRODUCTION

Ozone is a strong oxidant effective in controlling bacteria, molds, protozoa, and viruses (*1*). It was initially used as an alternative disinfectant to hypochlorite, and in 1997 it was reaffirmed as a GRAS product by an expert panel. Since then, the number of studies evaluating different uses in the food industry has rapidly increased (*2*). Ozone might have different applications, such as cleaning surfaces or equipment and disinfecting water for recycling (*3*). More recently, there has been interest in the evaluation of ozone treatments during processing and storage of fruits and vegetables (*4*−*7*). Continuous exposure to low concentrations (0.1−0.3 μL/L) in storage areas can oxidize ethylene (*8*), and treatments with ozone gas have been shown to elicit the accumulation of antioxidants (*9*). Achen and Yousef (*10*) reported that the use of ozone-containing water for washing apples decreased the counts of *E. coli* O157:H7. In addition to its potential effectiveness to control human pathogens, it has also been used to reduce the incidence and severity of spoilage-causing organisms (*4*, *5*). The effects of ozone, and its primary decomposition metabolites, are associated with multiple reactions, including the inactivation of enzymes, alteration of nucleic acids, and oxidation of membrane lipids (*1*). However, a higher inhibitory effect on mold development was observed when the treatments were done in inoculated fruit, relative to treatments of isolated fungi (*11*, *12*). This suggests that at least part of the decay control observed in ozonated produce is related to fruit-mediated responses and is consistent with previous works showing the activation of defensive pathways in ozone-treated plants (*13*). Treatments able to delay some ripening-associated processes may also reduce fruit susceptibility to physical damage and pathogen attack and contribute to decrease spoilage.

Different ozone treatments have been tested so far in tomato. Aguayo et al. (*14*) analyzed the effect of cyclic exposures to ozone (4 μL/L for 30 min every 3 h) in minimally processed fruit. The treatments delayed softening, improved some attributes associated with flavor (sugars, acids), and reduced the counts of total mesophilic bacteria and molds. A second type of treatments studied involved the continuous exposure of fruit atmospheres with low ozone concentrations (0.005−5 μL/L) (*11*, *12*). In this case, growth, sporulation, and decay caused by *Botrytis cinerea, Alternaria alternata*, and *Colletotrichum sp.* were reduced. Treated fruit also stayed firmer and presented higher acceptability in sensory analysis panels (*7*). Finally, some studies have evaluated the influence of short-term ozone exposure on fruits with promising results. Rapid immersion, sprays with ozonated water, and gaseous ozone treatments were effective in controlling *Salmonella* (*15*, *16*). The influence of these short-term ozone treatments on tomato fruit quality has received little attention to date. Previous works have showed that ozone treatments can reduce fruit softening, but the cell wall changes associated with these textural modifications have not been further characterized. In this work,

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we evaluated the effect of brief ozone exposure on tomato fruit quality as well as the changes in cell wall solubilization, depolymerization, and activity of some enzymes associated with pectic polysaccharide degradation.

MATERIALS AND METHODS

Plant Material, Ozone Treatments, and Storage. Greenhouse-grown tomato fruit (*Solanum lycopersicum* L.) at the light red ripening stage, produced in La Plata, Buenos Aires State, Argentina, were harvested and immediately transported to the laboratory. Fruit having blemishes or other defects were eliminated. Fruit was placed in a hermetic tray connected to an ozone generator (Dobzono, Model Ozolab 100, Argentina). Ozone was injected in order to reach a final concentration of 10 μL/L and held at that level for 5, 10, or 20 min. Ozone concentration was controlled with a sensor equipped with a semiconductor of metal oxides (DCMIV, International Xilix SA. Argentina). When the treatments were finished, air was blown through to remove ozone and was then bubbled in a 10% IK (w/v) solution to avoid ozone release to the atmosphere. Fruit was placed in plastic trays, covered with perforated PVC, and stored for 9 days at 20 °C (90% HR). Corresponding controls without ozone treatment were directly stored at 20 °C for 9 days. On the basis of the reduction of fruit damage (determined as the sum of fungal development plus wounding) the samples from the 10 min treatments were used for further analysis. A total of 45 control or ozone-treated fruit were removed after 0, 6, or 9 days and were directly used or were cut into 8 pieces, frozen in liquid nitrogen, and stored at −20 °C until used.

Fruit Damage. The presence of defects such as wounds, spots, and macroscopic fungal growth was visually evaluated. Results were expressed as a percentage of damaged fruit.

Total Phenols. Approximately 20 g of frozen fruit tissue was ground in a mill, and 1 g of the resultant powder was transferred to a tube containing 5 mL of ethanol (Mallinckrodt, Phillipsburg, NJ). The suspension was vortexed and then centrifuged at 17 000*g* for 10 min at 4 °C. The supernatant was collected, and the pellet was re-extracted with 5 mL of ethanol and centrifuged as described above. The supernatants were pooled and taken to 100 mL with distilled water. The extracts were used for total phenolic compound measurements according to Singleton and Rossi (*17*) with little modification. A 200 μL portion of 1 N Folin-Ciocalteu reagent (Anedra Bs As, Argentina) was added to 1.5 mL of the extract. After 3 min, 1.5 mL of a solution containing 20% (w/v) Na_2CO_3 in 0.1 N NaOH was added, and the mixture was incubated at 20 °C for 1 h. The absorbance at 760 nm was measured in a spectrophotometer (Beckman Model UV Mini-1240, Brea, CA), and total phenolic content was calculated by using phenol as standard. Two extracts were done at each sampling date for both control and treated fruit, and measurements were done in triplicate. Results were expressed as milligrams of phenol per kilogram of fresh weight.

Weight Loss. Individual fruit was weighed at the beginning of the experiment and during storage. Results were expressed as a percentage of weight loss relative to the initial weight.

Firmness. Firmness was measured by using a texture analyzer (TA. XT2, Stable Micro Systems Texture Technologies, Scarsdale, NY) fitted with an 8 mm flat probe. Each fruit was compressed 6 mm at the equatorial zone at a rate of 0.5 mm/s, and the maximum force developed during the test was recorded. Three measurements were done per fruit, and 20 independent control or ozone-treated fruits were analyzed for each storage time. Results were expressed in newtons.

Fruit Color, pH, Acidity, Sugars, and Antioxidant Activity. Surface color was measured with a colorimeter (Minolta, Model CR-400, Osaka, Japan). The parameters L^*, a^*, and b^* were obtained at the equatorial zone and used to calculate the hue angle (arctan b^*/a^*). Twenty measurements were done at each storage time analyzed for both control and ozone-treated fruit. For pH and acidity determinations, five fruit samples were ground and 10 g of the resulting slurry was suspended in 100 mL of distilled water. The pH of the sample was determined with a pH meter. Acidity was determined by titration (*18*) with 0.1 M NaOH (Anedra, Bs As, Argentina). Four independent measurements were done at each sampling date for both control and treated fruit. Results were expressed as milliequivalents of H^+ per kilogram of fresh fruit. For total sugar and antioxidant determinations, frozen fruit samples (1 g) were ground in a mill and the resultant powder was extracted with 5 mL of ethanol and centrifuged (at 17 000*g* at 4 °C for 10 min). The supernatant was collected, and a second extraction with 5 mL of ethanol was done. The supernatants were pooled and brought to 100 mL with distilled water. Total sugars were measured according to Yemm and Willis (*19*), and results were expressed as grams of glucose per kilogram of fresh fruit. Antioxidants were measured according to Brand-Williams et al. (*20*). The amount of extract required to react with 50% of the radical 2,2′-diphenylpicrylhydrazyl ($DPPH^•$) (Sigma-Aldrich, St. Louis, MO) after 60 min was determined and defined as EC_{50}. Results were expressed as $1/EC_{50}$ (1/g). Two extracts for sugars and antioxidants were done at each storage time for both control and treated fruits, and measurements were done in triplicate.

Respiration Rate. Samples of three fruits were put in a hermetic jar and incubated at 20 °C. The production of carbon dioxide was measured by using an IR sensor (ALNOR Compu-flow, Model 8650, Huntington Beach, CA). Fruit respiration rate was expressed as milliliters of CO_2 per kilogram-hour. Three independent measurements were done at every storage time analyzed for both control and ozone-treated fruit.

Isolation of Cell Wall Material and Determination of Alcohol-Insoluble Residue. Cell wall polysaccharides were isolated as previously described (*21*). The alcohol-insoluble residue (AIR) was weighed, and results were expressed as grams of AIR per 100 g of fresh fruit.

Cell Wall Neutral Sugars and Uronic Acids. Five milligrams of AIR was solubilized by adding 0.5 mL of 98% (w/w) H_2SO_4 in an ice bath and stirring for 10 min. This operation was repeated three times. After that, 500 μL of distilled water was added and the samples were stirred for 10 min. After dissolution, samples were brought to 10 mL with distilled water and used to analyze uronic acids (UA) and neutral sugars (NS). UA and NS were determined according to Blumenkrantz and Asboe-Hansen (*22*) and Yemm and Willis (*19*), respectively. Measurements were done in triplicate, and results were expressed for UA as milligrams of galacturonic acid per gram of AIR and for NS as milligrams of glucose per gram of AIR.

Pectin Solubilization. Polyuronides were isolated as described by Vicente et al. (*21*). Aliquots of 100 mg of AIR were suspended in 15 mL of distilled water and stirred at 20 °C for 12 h. The suspension was filtered through fiberglass, and the residue was washed three times with 10 mL of distilled water. The filtrate was designated as water-soluble fraction (WSF). The residue was then suspended in 15 mL of 50 mM sodium acetate buffer pH 5.0 containing 40 mM CDTA (Sigma-Aldrich, St. Louis, MO) and stirred for 12 h at 20 °C. The suspension was filtered through fiberglass, and the residue was washed three times with the same buffer. The filtrate was designated as CDTA soluble fraction (CSF). Finally, the residue obtained from the previous step was extracted with 20 mL of 50 mM Na_2CO_3 for 1 h at 4 °C. The suspension was filtered as described above, and the residue was washed three times with 10 mL of 50 mM Na_2CO_3 (Sigma-Aldrich, St. Louis, MO). The filtrate was designated as Na_2CO_3 soluble fraction (NSF) and the residue from pectin fractionation was saved for further extraction of hemicelluloses. UA and NS concentrations in all fractions were measured according to Blumenkrantz and Asboe-Hansen (*22*) and Yemm and Willis (*19*), respectively. Two independent extractions were done at time 0 and after 9 days of storage at 20 °C for both control and ozone-treated fruit, and each sample was measured in triplicate. Results were expressed as a percentage of WSF, CSF, or NSF relative to total extractable pectins.

Hemicellulose Solubilization. Depectinated AIR was stirred for 8 h at 4 °C with 20 mL of 4% KOH containing 1 mM sodium borohydride (Sigma-Aldrich, St. Louis, MO). The suspension was filtered and washed with 10 mL of 4% KOH, and the filtrate was designated as 4% KOH soluble fraction (4KSF). The same procedure was repeated on the residue with 24% KOH, to obtain the fraction soluble in this extractant (24KSF). Hemicellulose determinations were done following the procedure of Yemm and Willis (*19*). Two independent extractions were done at time 0 and after 9 days of storage at 20 °C for both control and ozone-treated fruit, and each sample was measured in triplicate. Results were expressed as milligrams of glucose per gram of AIR.

Pectin Depolymerization. Pectin polymer size distribution was done as previously described (*21*). Fractions from the NSF were dialyzed

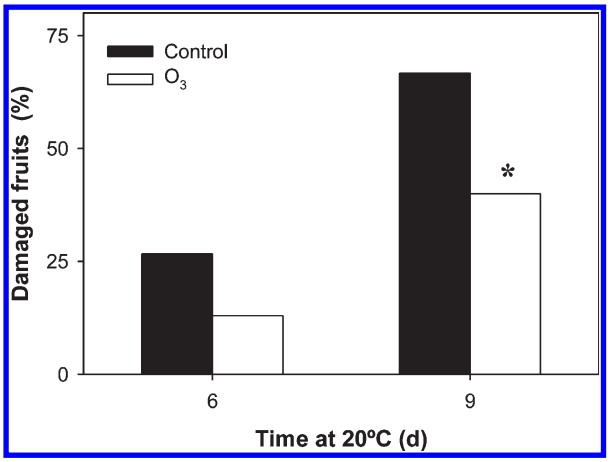

Figure 1. Fruit damage in control and ozone-treated tomatoes (O_3), and after 6 or 9 days of storage at 20 °C. The asterisks indicate significant differences from the corresponding control at a level of significance of $P \leq 0.05$.

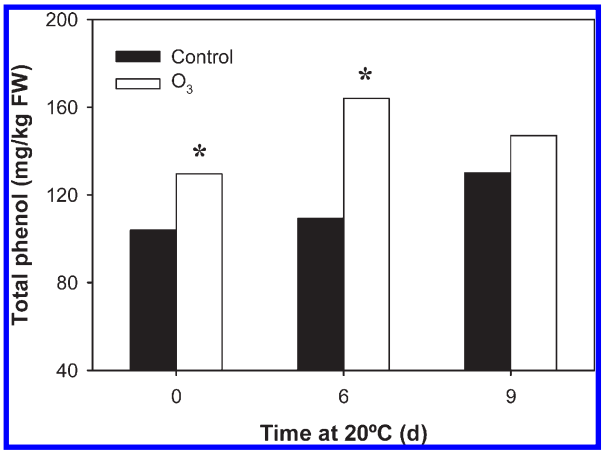

Figure 2. Total phenolics in control and ozone-treated fruit (O_3) immediately after treatment and after 6 or 9 days of storage at 20 °C. The asterisks indicate significant differences from the corresponding control at a level of significance of $P \leq 0.05$.

(Spectrapor 8 kD cutoff tubing, Spectrum Laboratories Inc., Rancho Dominguez, CA) against distilled water for 1 day at 4 °C, freeze-dried, and stored until use. Samples were suspended in 4 mL of 200 mM ammonium acetate (pH 5.5) and loaded in a SEC glass column (105 cm length; 1.8 cm diameter) packed with Sepharose Cl-6B (Pharmacia, Uppsala, Sweden). Ammonium acetate (200 mM, pH 5.5) was used as running buffer, at a flow rate of 0.9 mL/min, and 80 fractions (2.5 mL each) were collected. The samples were held in a water bath at 40 °C for 3 h to eliminate ammonium, which can interfere with uronic acid (UA) determinations. UA values were measured as previously described (*22*).

Polygalacturonase (PG) and β-Galactosidase (β-Gal) Activity. Approximately 5 g of frozen fruit was homogenized with 15 mL of sodium acetate buffer (50 mM, pH 5.5, 1 M NaCl, 10 g/L polyvinyl-polypyrrolidone; PVPP); the mixture was stirred for 2 h at 4 °C and then centrifuged at 17 000*g* for 10 min. The supernatant was dialyzed (Spectrapor 8 kD cutoff tubing, Spectrum Laboratories Inc., Rancho Dominguez, CA) against sodium acetate buffer (50 mM, pH 5.0) overnight at 4 °C, and the dialyzed samples were used to determine both enzyme activities. All the steps during the extract preparation were carried out at 0−4 °C. β-Gal activity was assayed in a mixture containing 800 μL of 50 mM sodium acetate buffer pH 5.0, 200 μL of 3 mM *p*-nitrophenyl β-D-galactopyranoside (ICN Biomedicals, Solon, OH) and 500 μL of enzymatic extract. The reaction mixture was incubated at 37 °C, and aliquots of 200 μL were taken at different times and discharged into 500 μL of 0.4 M Na_2CO_3. The change of OD at 410 nm was measured with a spectrophotometer (Beckman Model UV Mini-1240, Brea, CA), and results were expressed as ΔOD in 1 s under the assay conditions per kilogram of fresh fruit.

PG activity was measured in a mixture containing 50 mM sodium acetate buffer pH 5.0, 0.15% (w/v) polygalacturonic acid, and 1 mL of enzymatic extract, in a total volume of 3 mL. The mixture was incubated at 37 °C, aliquots of 300 μL were taken at different times, and the galacturonic acid released was measured with 2-cyanoacetamide (Sigma-Aldrich, St. Louis, MO) according to Gross (*23*). Results were expressed as micrograms of galacturonic acid released in 1 sunder the assay conditions per kilogram of fresh fruit. Two independent extracts were done at time 0 and after 9 days at 20 °C for both control and ozone-treated fruit, and each extract was measured twice.

Pectin Methylesterase (PME) Activity. Five grams of frozen fruit was ground with 15 mL of 1 M NaCl and 10 g/L PVPP. The suspension obtained was stirred for 4 h and then centrifuged at 17 000*g* for 30 min. The supernatant was collected, adjusted to pH 7.5 with 0.01 M NaOH, and used for assaying the enzyme activity. All the steps during the extract preparation were carried out at 0−4 °C. The activity was assayed in a mixture containing 1 mL of 0.5% (w/v) pectin (Sigma-Aldrich, St. Louis, MO), 400 μL of 0.01% (w/v) bromothymol blue pH 7.5, 1.55 mL of distilled water adjusted to pH 7.5, and 50 μL of enzymatic extract. The mixture was incubated at 37 °C, and the reduction of OD at 620 nm was followed. Results were expressed as ΔOD in 1 s under the assay conditions per kilogram of fresh fruit. Four independent extracts were done at time 0 and after 9 days at 20 °C for both control and ozone-treated fruit, and each sample was measured twice.

Statistical Analysis. Experiments were performed according to a factorial design. Angular transformation for damaged fruit was performed prior to statistical analysis. Data were analyzed by ANOVA, and the means were compared by a Fisher test at a significance level of 0.05.

RESULTS AND DISCUSSION

Fruit Damage. The percentage of damaged fruit (as the sum of fungal development plus wounding) increased during storage at 20 °C. Previous works have reported the temporary effects of ozone. Instead, in this study, differences seemed to be clearer at the end of the storage period when the fruit was fully red. Ozone exposure resulted in a 27% reduction of damaged fruits after 9 days of storage at 20 °C (**Figure 1**). Treatments for 5 min also reduced fruit damage, but the effect was less dramatic. Exposure to ozone for 20 min resulted in control of fungal pathogens similar to that observed with 10 min treatments (data not shown). Consequently the 10 min treatments were used to further characterize the effect on fruit quality and cell wall metabolism.

Previous studies reported that ozone treatments might be useful to reduce postharvest decay (*11*, *12*). However, the treatments tested involved the exposure to ozone for several days. Cyclic treatments have been effective to maintain quality and reduce microbial populations in whole and sliced tomato (*14*). Short ozone exposures have been also evaluated in tomato, but focusing mainly on the potentiality to control human pathogens (*15*, *16*).

Phenolic Compounds. The inactivation of microorganisms by ozone is complex, because this compound can directly react with different cellular constituents, such as membrane lipids, causing membrane dysfunction or even cell lysis, enzymes, altering metabolic homeostasis, and/or nucleic acids, disrupting gene expression and cell division (*1*−*3*). In addition to the direct effect of ozone on molds and bacteria, it has been suggested that it can trigger the accumulation of phytoalexins or activate other defense mechanisms (*12*, *13*, *24*). In the present work, we found that ozone treatments induced the accumulation of phenolic compounds (**Figure 2**). The rapid response observed suggests that the modifications might have been due to the activation of pre-existing enzymes. However, during storage the differences in the content of phenolic compounds increased, and after 6 days at 20 °C ozone-treated tomatoes presented a 50% increase relative to

Table 1. Respiration Rate, Weight Loss, Lightness, Surface Color (Hue), Acidity, pH, Sugars, and Antioxidant Capacity in Control (C) and Ozone-Treated Fruit (O_3) Immediately after Treatment and after 6 or 9 Days of Storage at 20 °C[a]

		storage time at 20 °C			
		0	6 days	9 days	LSD
respiration (mL of CO_2/(kg h))	C	16.7	11.7	10.5	
	O_3	19.3	13.5	10.6	
					2.6
weight loss (%)	C	0	1.2	6.1	
	O_3	0	1.3	5.5	
					0.4
fruit lightness (L^*)	C	47	42	41	
	O_3	47	42	41	
					6.1
surface color (hue)	C	69	53	53	
	O_3	74	55	53	
					6.9
acidity (mequiv of H^+/kg of FW)	C	57.2	50.7	46.3	
	O_3	59.2	51.9	46.2	
					3.98
pH	C	4.46	4.52	4.59	
	O_3	4.40	4.53	4.50	
					0.05
sugar (g/kg of FW)	C	24.4	22.9	23.5	
	O_3	23.0	22.0	22.0	
					2.5
antioxidant capacity $1/EC_{50}$ (1/g FW)	C	49	52	52	
	O_3	43	57	51	
					8

[a] The least significant difference (LSD) at $P \leq 0.05$ is indicated.

control fruit. Booker and Miller (*25*) found that ozone treatments induced phenylalanine ammonia-lyase (PAL), a key regulatory enzyme in the biosynthesis of phenolic compounds, and resulted in increased accumulation of caffeic and *p*-coumaric acid (*25*). The correlation between phenolic compounds and reduced fruit damage might favor hypotheses related to the involvement of fruit defense responses in preventing the spread of fungal pathogens. However, other modifications, induced by ozone, could have contributed to the reduction of damaged fruit observed during storage.

After 9 days of storage the content of phenolic compounds increased in control fruit (20%) and showed a slight reduction (10%) in ozone-exposed tomatoes. The accumulation of phenolics toward the end of the storage period in the controls might have been related to the higher damage found (**Figure 1**). With regard to the reduction detected in ozone-treated fruit, cross-linkage of phenolic compounds could have reduced soluble fractions and contributed to reinforce the cell walls. However, this remains speculative, and further studies are required to address this issue.

Respiration, Weight Loss, Color, pH, Acidity, Sugars, and Antioxidant Capacity. Results in respiration rate showed a transient increase immediately after the treatment, but no differences were found between control and treated fruit afterward (**Table 1**). After 2 days of storage no difference in fruit color or weight loss were observed between control and ozone-treated tomatoes (data not shown). Fruit weight loss increased during storage in both control and treated fruit. After 9 days at 20 °C, ozone-exposed tomatoes showed reduced weight loss.

Postharvest treatments aimed in extending fruit storage capacity should not alter flavor and nutritional quality. The ozone treatments employed in this work did not cause significant modifications in titratable acidity or sugars. No alterations in fruit antioxidant capacity were observed either (**Table 1**).

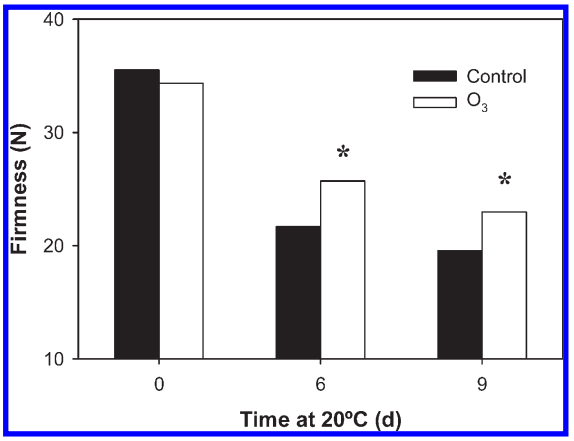

Figure 3. Firmness in control and ozone-treated fruit (O_3) immediately after treatment and after 6 or 9 days of storage at 20 °C. The asterisks indicate significant differences from the corresponding control at a level of significance of $P \leq 0.05$.

Firmness. Tomato fruit softening is a major modification occurring during ripening and storage, resulting in increased susceptibility to physical damage and fungal attack (*26*). Fruit firmness was not affected immediately after the treatments (**Figure 3**). The average softening rate during the first 6 days of storage was 2.3 N per day for control fruit and 1.4 N per day for ozone-treated fruit. Consequently, ozone-treated fruit was significantly firmer than control fruit and the differences remain until the end of the storage period. Similar results were reported by Aguayo et al. (*14*) in minimally processed tomatoes cyclically treated with ozone. Other studies have also described that ozone exposure resulted in better firmness retention (*7*). However, the ozone concentrations and treatment durations (several days) in these cases were quite distinct from those tested herein. Results from the present work show that short-term treatments with ozone (10 μL/L) are effective in reducing tomato fruit softening. Most works performed evaluating ozone treatments and fruit textural modifications have been limited to describe the changes in firmness, without analyzing this phenomenon further. In this sense, we decided to start studying the effect of the ozone treatments on cell wall modifications that could be related to the delay in softening observed.

Total Cell Wall Composition. It is known that cell wall degradation has a main role in the rheological properties of fruits (*27*). We isolated the tomato cell walls as alcohol-insoluble residue (AIR) and started characterizing the changes observed in response to ozone treatments. Total AIR represented around 1.3−1.4% of fruit fresh weight, but no differences between treatments were found during storage (**Table 2**). The contents of uronic acids and neutral sugars in the AIR were around 40 and 50%, respectively, and similar levels were found for control and treated fruit throughout the storage period (**Table 2**).

Pectin and Hemicellulose Solubilization. In order to further characterize the cell wall from control and ozone-treated fruit, we fractionated pectin and hemicelluloses. The content of hemicelluloses in 4KSF and 24KSF was 35 and 55 mg/g of AIR, respectively. No differences were found in hemicellulose content, either between control and treated fruit or during storage (data not shown). Pectic polymers were extracted on the basis of their solubility in water (WSF), CDTA (CSF), or Na_2CO_3 (NSF) representing the polyuronides loosely, ionically, or tightly associated to the wall (*28*). At the beginning of the storage period, WSF accounted for approximately 30% of total uronic acids in both control and ozone-treated fruit (**Figure 4A**). After 9 days of

Table 2. Changes in Alcohol-Insoluble Residue (AIR), Proportion of Cell Wall Uronic Acids, and Neutral Sugars in Control (C) and Ozone-Treated Fruit (O_3) Immediately after Treatment and after 9 Days of Storage at 20 °C[a]

		storage time at 20 °C		
		0	9 days	LSD
AIR (g/100 g of FW)	C	1.4	1.4	
	O_3	1.4	1.3	
				0.2
neutral sugars (mg/g of AIR)	C	419	502	
	O_3	429	474	
				53
uronic acids (mg/g of AIR)	C	342	383	
	O_3	324	320	74

[a] In each case, the LSD at $P \leq 0.05$ is indicated.

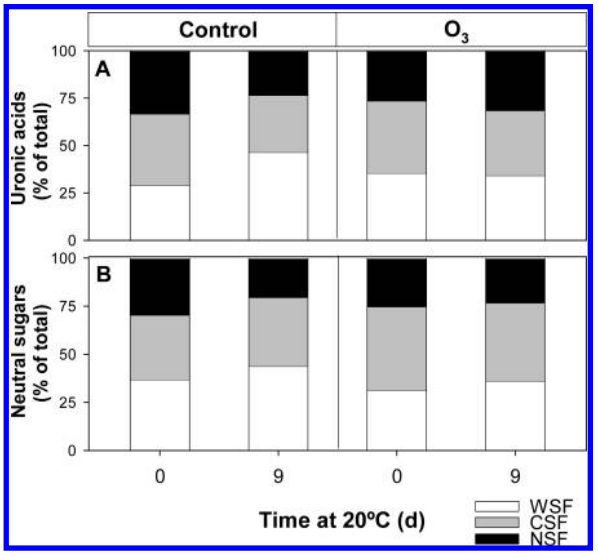

Figure 4. Change in the proportion of pectin solubilized in water (WSF), CDTA (CSF), and Na_2CO_3 (NSF) in control and ozone-treated fruit (O_3), immediately after treatment and after 6 or 9 days of storage at 20 °C: (**A**) uronic acids; (**B**) neutral sugars. $LSD_A = 9.8$, $LSD_B = 7.1$.

storage at 20 °C the percentage of total uronic acids extractable in water increased to 50% in control fruit but remained in 30% in fruit exposed to ozone. This increase occurred with the concomitant decrease of the polyuronides tightly associated to the cell wall. When neutral sugars were measured in these fractions, the same trend was found, but in this case the differences were less marked (**Figure 4B**). Results show that ozone treatments reduce pectin solubilization and that the effect is higher in uronic acids than in neutral carbohydrates. In contrast, no changes occur in hemicellulosic polysaccharides.

Pectin Depolymerization. Pectin depolymerization is another change typically observed during tomato fruit ripening, which can also contribute to increase the solubility and ease of extraction of wall polyuronides. **Figure 5** shows the size exclusion profiles from control and ozone-treated tomato fruit after 9 days of storage. Results show that fruit exposed to ozone maintained higher pectin mean molecular size.

Pectin-Degrading Enzymes. One of the main modifications in cell wall sugars accompanying tomato fruit ripening is the loss of galactose (*29*). This has been associated with increased solubilization of pectins. The cleavage of galactose-rich polysaccharides such as rhamnogalacturonan I (RG I) has been linked to the action of the hydrolytic activity of β-Galactosidase (β-Gal). The activity of β-Gal decreased during storage, but no differences were found between control and treated fruit (**Figure 6B**),

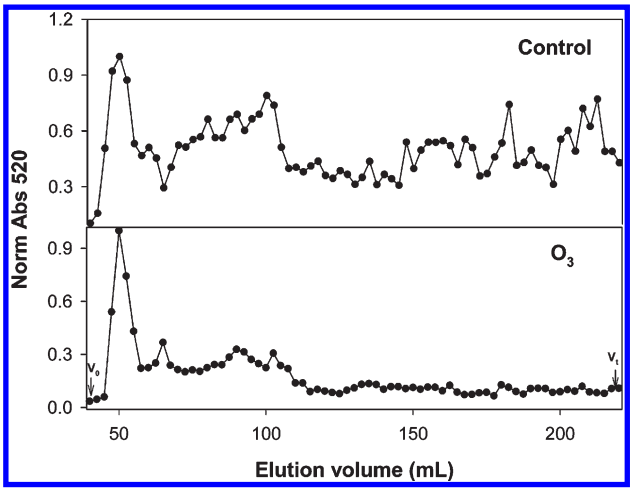

Figure 5. Size exclusion chromatography of the Na_2CO_3-soluble pectins (NSF) from control and ozone-treated tomato fruit (O_3), stored at 20 °C for 9 days, fractionated on Sepharose CL-6B. Column fractions (2.5 mL) were assayed for uronic acid content using the *m*-hydroxybiphenyl method (*22*). V_0 denotes void volume, and V_t denotes total volume.

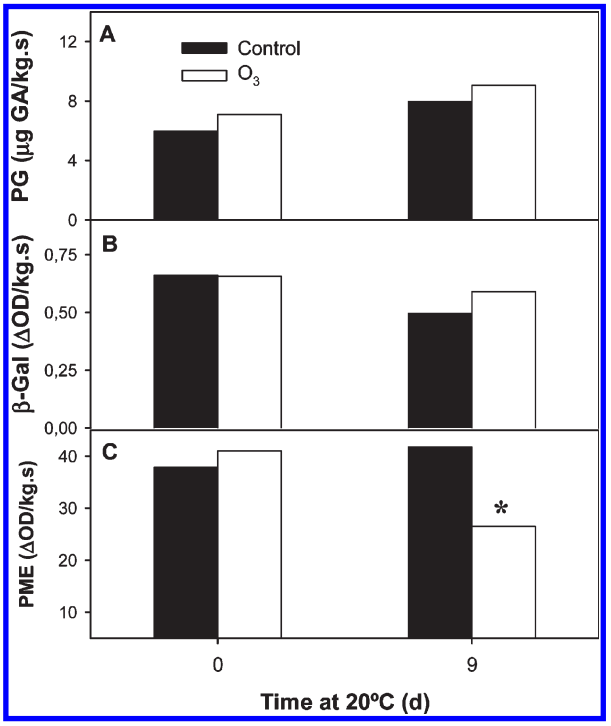

Figure 6. (**A**) Polygalacturonase (PG), (**B**) β-galactosidase (β-Gal), and (**C**) pectin methylesterase (PME) activity in control and ozone-treated fruit (O_3) immediately after treatment and after 6 or 9 days of storage at 20 °C. The asterisk indicates significant differences from the corresponding control at a level of significance of $P \leq 0.05$.

suggesting that the differential solubilization of pectins observed is not related to changes in this enzyme. No significant differences in PG activity were detected between control and ozone-treated fruit either (**Figure 6A**). Another enzyme involved in pectin metabolism is pectin methylesterase (PME). Although immediately after the treatments no differences were detected between control and treated fruit, after 9 days of storage ozone-treated fruit showed a 50% reduction in PME activity relative to control fruit (**Figure 6C**). Rico et al. (*30*) also found that PME activity decreased upon ozone treatments in lettuce. In tomato, pectin esterification decreases from 90% to 30% during ripening and

demethylated polyuronides are more susceptible to be cleaved by PG (*31*). Thus, reduction in PME might have modulated pectin depolymerization. However, the modification in other cell wall degrading enzymes or changes in cell wall cross-linkages might have also contributed to modify the disassembly of fruit cell walls. For instance, the differences observed in texture might be associated with changes in the activity of other pectin depolymerizing agents such as pectate lyase (PL). Other changes that could occur in ozone-treated tissues include the cross-linking of cell wall structural proteins by formation of dityrosine associations (*32*). These associations have been shown to reduce the solubility and strengthen the cell wall (*33*).

The complexity of the modifications induced by exposure of fruits to ozone is far from being understood. In this work we found that brief ozone treatment (10 μL/L; 10 min) reduced damage and softening of tomato fruit during storage. While exposure to ozone elicited the accumulation of phenolic compounds which might have antimicrobial properties, it also caused a clear decrease of PME activity, pectin solubilization, and depolymerization. The lower disassembly of cell wall polyuronides might be an important contributor to the reduced softening and damage of ozone-treated fruits.

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