

# Food Science and Technology International

<http://fst.sagepub.com/>

---

## **Quality of tomato slices disinfected with ozonated water**

Encarna Aguayo, Víctor Escalona, Ana Cecilia Silveira and Francisco Artés

*Food Science and Technology International* 2014 20: 227 originally published online 17 June 2013

DOI: 10.1177/1082013213482846

The online version of this article can be found at:

<http://fst.sagepub.com/content/20/3/227>

---

Published by:



<http://www.sagepublications.com>

On behalf of:



[Consejo Superior de Investigaciones Científicas \(Spanish Council for Scientific Research\)](#)

**Additional services and information for *Food Science and Technology International* can be found at:**

**Email Alerts:** <http://fst.sagepub.com/cgi/alerts>

**Subscriptions:** <http://fst.sagepub.com/subscriptions>

**Reprints:** <http://www.sagepub.com/journalsReprints.nav>

**Permissions:** <http://www.sagepub.com/journalsPermissions.nav>

>> [Version of Record](#) - Mar 21, 2014

[OnlineFirst Version of Record](#) - Jun 17, 2013

[What is This?](#)



# Quality of tomato slices disinfected with ozonated water

Encarna Aguayo<sup>1,2</sup>, Víctor Escalona<sup>3</sup>, Ana Cecilia Silveira<sup>4</sup> and Francisco Artés<sup>1,2</sup>

## Abstract

Fresh-cut industry needs novel disinfectant to replace the use of chlorine. Ozone is one of the most powerful oxidizing agents and is applied in gaseous or aqueous form for sanitation purposes. However, the strong oxidative effect could affect the nutritional and sensorial quality, in particular, when time of washing is extended. For that reason, the overall impact of ozonated water (0.4 mg/L) dipping applied during 1, 3 and 5 min compared to control washed in water during 5 min was studied in tomato slices stored during 14 days at 5 °C. According to the results, ozonated water treatment of 3 min achieved the best firmness retention, microbial quality (mesophilic, psychrotrophic and yeas load) and reduced the consumption of fructose and glucose. The use of ozonated water did not affect the total acidity, pH, total solid soluble, organic acid as ascorbic, fumaric or succinic acid and the sensorial parameters, which were only affected by storage time. However, the poor appearance, aroma and overall quality obtained in all treatments prevented shelf life of 14 days and the quality at acceptable levels was established in 10 days at 5 °C. It is recommended to wash tomato slices with 0.4 mg/L ozonated water for 3 min only. Extending treatment duration did not improve the microbiological quality, possibly due to the extra time permitting the ozone to react with other components of the fruit tissue, undermining the antimicrobial benefits.

## Keywords

Respiration rate, ethylene, microbiological growth, fresh-cut, ozone

Date received: 4 October 2012; revised: 7 January 2013

## INTRODUCTION

Tomato (*Lycopersicon esculentum* L.) is one of the most widely consumed vegetables, being the second most important vegetable crop worldwide. It is a key component in the so-called 'Mediterranean diet', and is noted for its high content of lycopene which has up to twice the antioxidant activity of  $\beta$ -carotene (Bramley, 2000). Due to their widespread consumption and nutritional interest, the industry has developed various tomato-based products as fresh-cut. During the fresh-cut elaboration process, a series of unit operations such as peeling, cutting, washing and disinfection etc. are carried out. As a result of this, a large number of physiological phenomena, such as biochemical

changes and microbiological spoilage, take place and may result in degradation of color, texture and flavor (Martín-Belloso et al., 2006). Along the processing line, the washing and disinfection processes are critical for the removal or inactivation of pathogens and is traditionally carried out using chemical products, especially chlorine derivatives (Delaquis et al., 2004). However, chlorine derivatives are associated with the production of chlorinated organic compounds such as trihalo-methanes, which are potential carcinogens, and also with the production of high amounts of wastewater

Food Science and Technology International 20(3) 227–235

© The Author(s) 2013 Reprints and permissions:

sagepub.co.uk/journalsPermissions.nav

DOI: 10.1177/1082013213482846

fst.sagepub.com



<sup>1</sup>Postharvest and Refrigeration Group, Universidad Politécnica de Cartagena (UPCT), Spain

<sup>2</sup>Instituto de Biotecnología Vegetal (UPCT), Spain

<sup>3</sup>Center of Postharvest Studies, Universidad de Chile, Chile

<sup>4</sup>Vegetable Production Department, Postharvest Division, University of the Republic, Montevideo, Uruguay

### Corresponding author:

Encarna Aguayo, Paseo Alfonso XIII, 44. E-30203, Cartagena, Spain.

Email: encarna.aguayo@upct.es

with very high levels of biological oxygen demand (Ölmez and Kretzschmar, 2009).

This scenario has created the need to investigate the efficiency of non-traditional sanitizers such as ozone, a three-atom molecule (triatomic allotrope) of oxygen ( $O_3$ ), formed from dry air (water in some cases) when diatomic oxygen ( $O_2$ ) is broken apart into free radicals ( $O^*$ ) that bond with diatomic oxygen forming  $O_3$  (Khadre et al., 2001).  $O_3$  is one of the most powerful oxidizing agents (oxidizing potential of 2.07 V compared to 1.36 V of chlorine) used in the food industry, and is applied in gaseous or aqueous form for sanitation purposes (Güzel-Seydim et al., 2004; Kim et al., 1999). As an aqueous disinfectant, declared to be generally recognized as safe (GRAS) for food contact application, it has been suggested as an interesting alternative to traditional chlorine-based sanitizers, due to its efficacy at low concentrations, short contact times, and its breakdown to non-toxic products (Graham, 1997). The effectiveness of  $O_3$  as an aqueous sanitizer has been tested in different products like lettuce leaves (Koseki and Isobe, 2006) using 3, 5 and 10 mg/L for 5 min at ambient temperature or in shredded lettuce using ozonated water at 5 mg/L for 5 min (Selma et al., 2007). Silveira et al. (2010) treated fresh-cut 'Galia' with concentration of 0.4 mg/L during 3 min, and more recently, Alexandre et al. (2011) on washed peppers with ozonated water at 2 mg/L for 3 min. Because of its strong oxidant characteristics,  $O_3$  also affects physiological processes such as respiration and ethylene production (Aguayo et al., 2006; Skog and Chu, 2000), enzymatic browning (Beltrán et al., 2005; Kim et al., 1999; Koseki and Isobe, 2006; Rico et al., 2006) color changes and other components (Artés-Hernández et al., 2007).

In this sense, the objective of this work was to study the influence of the disinfected treatments using ozonated water for different times in the microbiological and overall quality of tomato slices preserved at 5 °C under modified atmosphere packaging (MAP) conditions.

## MATERIAL AND METHODS

### Plant material

Tomatoes of the 'Thomas' variety were grown in a greenhouse of the Mediterranean region of Mazarrón (Murcia, Spain), and manually harvested in June. Tomatoes were commercially ripened fruit, at stage 8 according to the Kleur-Stadia (Holland) tomato color chart. These tomatoes were not washed in the packing house as it is usually done with those intended for export before going through the sorting. The fruits were selected based on their size and external color, discarding those with injuries from bumps and/or abrasions or

postharvest rots. In a sample of 20 tomatoes taken at random, the mean values and standard deviations of weight ( $105.8 \pm 1.2$  g), equatorial ( $3.5 \pm 0.1$  cm) and longitudinal ( $4.0 \pm 0.1$  cm) diameters were monitored. In this sample, skin color was determined with a colorimeter and expressed as CIELAB parameters, being  $L^* = 55.42 \pm 0.5$ , Hue angle ( $h^\circ$ , arctangent  $b^*/a^*$ ) =  $52.14 \pm 1.3$  and Chroma ( $C^*$ ,  $[(a^*)^2 + (b^*)^2]^{0.5} = 22.08 \pm 0.4$ ).

### Sample preparation, treatments and storage conditions

Four random lots of about 10 kg of fruit each were formed. Subsequently, the tomatoes were cut with an automatic slicer machine (RG-100 Hallden, Sweden) obtaining slices of  $0.8 \pm 0.1$  cm thick (perpendicular to the polar axis). Both the machine and the tools used were previously disinfected with a solution of NaOCl (100 mg/L). The tomato slices were washed in a tank which contained ozonated water (8 °C and pH=7) during 1, 3 and 5 min. The continuous ozonated water was provided by an  $O_3$  generator (Cosemar ozono, Madrid, Spain), at a concentration of 0.4 mg/L. The indigo trisulfonate spectrophotometric method was used for calibration and also to check the applied  $O_3$  concentration (APHA, 1998; Silveira et al., 2010).

The water was changed after the different treatments. Control treatment corresponded to washing for 5 min in the same tank but using tap water (without  $O_3$ ). Treated tomato slices were drained using a colander and placed in trays with filter paper to remove the excess water. About 250 g of sliced tomatoes were packaged into polypropylene (PP) trays that were heat-sealed (Barket, Befor Model, Chassieu, France) on the edges with a micro-perforated oriented polypropylene film (OPP) of 35  $\mu$ m thickness (Danisco Flexible, Bristol, UK), with fog treatment. Five replicates per treatment were prepared. After sealing, the trays were transferred to a cold room at 5 °C and stored for 14 days at 5 °C and 95% RH. Evaluations were made at the beginning of the experiment (day 0) and on days 5, 10 and 14.

### Respiration rate and ethylene emission

Respiration rate and ethylene ( $C_2H_4$ ) emission were determined by gas chromatography comparing the control treatment (washed with water) and the disinfection with ozonated water during 5 min. Samples of about 150 g of tomato slices were placed in 1 L hermetic glass jars connected to a gas flow panel (Flowboard, Davis, CA, USA) with an air flow of 0.1–0.2 L/h, humidified to 95% RH. The jars were provided with a silicone septum in the lid, through which gas samples (0.5 mL) were taken, with a plastic syringe, after 2 h of closure.

This sample was injected in a gas chromatograph (GC) (Shimadzu GC-14, Tokyo, Japan) equipped with a thermal conductivity detector (TCD) at 200 °C, using a column Chromosorb 102 (2 m × 1/8" SS Supelco, Inc., Bellefonte, Penn, USA). Oven and injector temperatures were 50 and 100 °C, respectively. The measurements were done every 1 or 2 days during 14 days at 5 °C. Between measurements, jars were flushed with humidified air flow in order to avoid excess of CO<sub>2</sub> accumulation (>0.3 kPa). To determine the C<sub>2</sub>H<sub>4</sub>, samples were taken following the same methodology described for the respiration rate, using a plastic syringe of 0.5 mL and injected the gas sample into a gas chromatograph (Hewlett Packard 5730A, USA) equipped with a flame ionization detector (FID) and a column of 1.20 m × 3.18 mm (Porapak QN 80/100, Norwalk, Connecticut, USA).

### Gas composition measurement

O<sub>2</sub> and CO<sub>2</sub> inside the trays were determined taking 0.5 mL gas sample with a plastic syringe through a silicone septum placed on the plastic film. These samples were analyzed using a GC (Perkin Elmer Autosystem, Norwalk, Connecticut, USA) equipped with a TCD.

### Microbiological growth

Three random samples of 30 g per treatment were homogenized with 270 mL of sterile peptone water (Merck, Darmstadt, Germany) for 1 min in a sterile bag (Model 400 Bags 6041, London, UK) using a masticator (Colwort Stomacher Lab 400, Seward Medical, London, UK). Serial dilutions were prepared as needed for plating. Under aseptic conditions, aliquots of 1 mL were extracted for aerobic mesophilic and psychrotrophic bacteria counting on plate count agar (PCA, Merck, Darmstadt, Germany), while molds and yeasts were counted using aliquots of 0.2 mL plated on rose bengal medium (RBM, Merck, Darmstadt, Germany). Incubation conditions were 30 °C for 48 h for mesophilic bacteria and yeast, 7 °C for 5 days for psychrotrophic bacteria and 22 °C for 5 days for molds. Total counts were expressed in log<sub>10</sub> colony forming units per gram (cfu/g). Microbiological quality was evaluated considering the maximum required for fresh-cut products industry in Spain (RD 3484/2000, 2001), which sets a maximum of 7 log cfu/g for aerobic bacteria, 5 log cfu/g for yeast and 3 log cfu/g for molds.

### Firmness measurement

The flesh firmness was determined based on the resistance to the deformation by compressing the tomato slices using a texture analyzer provided with two

plates (12 × 18 cm) at a rate of 4 mm/min (Aguayo et al., 2006). The results were expressed in Newton (N).

### Physicochemical parameters

Each repetition was crushed with a domestic blender (Moulinex, Barcelona, Spain) and the juice obtained analyzed to determine the total soluble solids (TSS), pH and titratable acidity (TA). TSS was determined with a refractometer (Atago N1, Tokyo, Japan) at 20 °C expressing the results in °Brix. The pH was determined using a pH meter (Crison 501, Barcelona, Spain) while the TA was determined by titration of 10 mL of juice with NaOH 0.1 N to pH 8.1 (Aguayo et al., 2006) expressing the results as g of citric acid per 100 mL.

For organic acids (citric, malic, succinic, ascorbic and fumaric acid) and sugars (fructose and glucose) determination the juice was filtered using cheesecloth and then centrifuged at 10,468 g during 15 min (Sigma 1-13, Germany). The supernatant was filtered using a nylon filter of 45 μm porosity (Whatman, Clifton NJ, USA) and subsequently using a Sep-Pack (C-18 Cartridges Waters, Taunton, Ireland). For sugar and organic acid determination, aliquots of 20 and 10 μL, respectively were injected into an HPLC (Merck Hitachi, Darmstadt, Germany) according to the methodology described by Aguayo et al. (2006).

### Color measurement

The color measurement was made on the tomato slice juice using a compact tri-stimulus colorimeter (Minolta CR-300, Ramsey NJ, USA) with an aperture diameter of 8 mm, previously calibrated with a white calibration plate ( $Y = 94.3$ ,  $x = 0.3142$ ,  $y = 0.3211$ , illuminant C and 2° observer) measuring L\*a\*b\* parameters in the CIE scale and calculating hue angle ( $h^\circ = \arctg b^*/a^*$ ) and chroma ( $C^* = [(a^{*2} + b^{*2})^{0.5}]$ ).

### Sensory evaluation

A panel of eight trained people (four men and four women; aged 25–55) analyzed visual appearance, aroma, taste and texture using the same methodology described by Aguayo et al. (2006). Evaluations were scored based on a nine-point scale (1 = extremely poor, 3 = poor, 5 = acceptable, limit of marketability, 7 = good and 9 = excellent). The overall appreciation of a sample was measured on the same scale and referred as overall quality.

### Statistical analysis

In firmness, physicochemical parameters, color measurement and sensory evaluation, the interactions

between storage time and disinfection treatments were studied by applying a bi-factorial ANOVA (Table 1). When differences among treatments were significant, the mean values were compared by the least significant difference (LSD) multiple range test using the Sigma Stat program.

## RESULTS AND DISCUSSION

### Respiration rate and ethylene emission

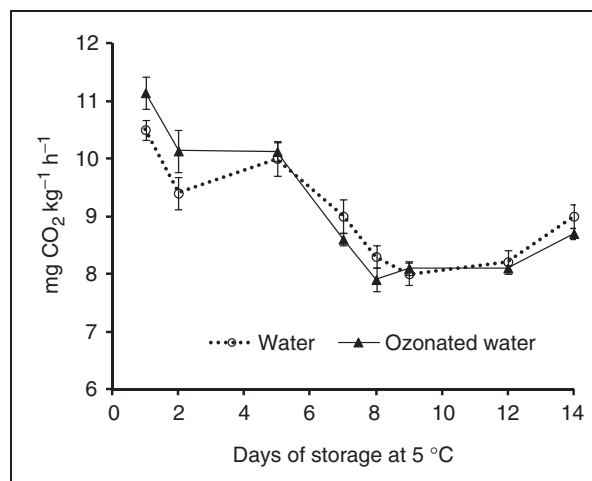
A high initial respiration rate, resulting of the stress generated during cutting and washing, was observed both in control and in ozonated water tomato slices washed for 5 min, being that the O<sub>3</sub> promoted a little more stress (Figure 1). This behavior was found in a previous work where O<sub>3</sub> gas (4 ± 0.5 µl/L of O<sub>3</sub> for 30 min every 3 h) stimulated the respiration rate of whole and fresh-cut tomato during the first 2 days of storage, after this period, the respiration of the treated ones was lower than the control (Aguayo et al., 2006). From day 5 to 12, both treatments reduced and stabilized, only to increase again slightly until day 14, consequence of decay. The effect of the O<sub>3</sub> treatment on the respiration rate on fresh-cut products is known to be quite variable. In some cases, O<sub>3</sub> reduces the respiratory rate as occurred on fresh-cut celery washed with ozonated water (0.08 and 0.18 mg/L during 5 min) (Zhang et al., 2005), in ‘Galia’ melon pieces washed with ozonated water (0.4 mg/L) during 3 min (Silveira et al., 2010) or in the case of carrots sticks treated with 10 mg/L during 10 min (Chauhan et al., 2011). However, Martínez-Sánchez et al. (2007) mentioned that the ozonated water (10 mg/L, 1 min at 8 °C) did not affect the respiration rate of rocket leaves.

The C<sub>2</sub>H<sub>4</sub> emission showed a very similar trend to that observed on the respiration rate. Initially, both treatments, the disinfection with ozonated water during 5 min and the control, showed the stress of the cutting accompanied by increased levels of C<sub>2</sub>H<sub>4</sub> being a little more intense in the case of the tomato slices disinfected with O<sub>3</sub>, as observed on Figure 2.

From day 2, both treatments reduced the C<sub>2</sub>H<sub>4</sub> emission and after 9 days of storage, tomato slices washed in ozonated water maintained lower C<sub>2</sub>H<sub>4</sub> production, a fact that could be linked to an indirect consequence of the microbial contamination in the water control treatments.

### Gas composition

As expected, the use of a micro perforated film prevented O<sub>2</sub> reduction and CO<sub>2</sub> accumulation, enabling the O<sub>3</sub> effect study. Due to the similarity in respiratory rates in both treatments, the gas composition of the packages were similar, reaching values of about 15.6 ± 0.3 kPa O<sub>2</sub> and 1.5 ± 0.2 kPa CO<sub>2</sub> (data not shown). The C<sub>2</sub>H<sub>4</sub> levels were between 5 and 5.6 mg/L (data not shown), and no differences between treatments were observed, except in the early days where the trays with the tomato slices disinfected with ozonated water for 5 min reached a slightly higher



**Figure 1.** Respiration rate of ‘Thomas’ sliced tomato washed in water or in ozonated water (0.4 mg/l) during 5 min and stored 14 days at 5 °C. Means (n = 5) ± SE.

**Table 1.** Statistic effect of parameters studied according to the simple factors as time of storage and washing treatment and the interaction between them through analysis of variance (p < 0.05)

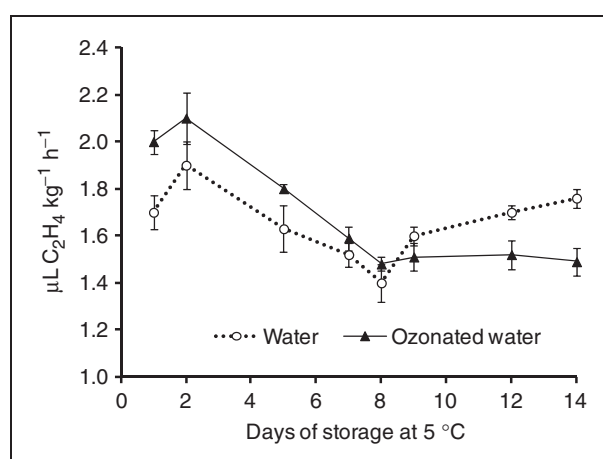
	Firmness	TA	pH	TSS	Organic acids			Ascorbic	Fumaric	Monosaccharides		Color			Sensorial
					Citric	Malic	Succinic			Glucose	Fructose	L*	h°	C*	Parameters
Time of storage	S	S	S	S	NS	NS	S	S	S	S	S	NS	NS	S	
Washing treatment	NS	NS	NS	NS	NS	NS	NS	NS	NS	S	S	NS	NS	S	
Time x washing	S	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	

S: significant effect; NS: not significant effect; TA: total acidity; TSS: total solid soluble; L\*: luminosity; H°: hue angle; C\*: chroma. In Tables 3 to 5 and Figure 3, mean values were compared by LSD (multiple range least significant difference test) when significant differences was found.

concentration, which can be attributed to the higher initial stress as mentioned before.

### Microbiological growth

The effect of the ozonated water on the microbiological growth is shown in Table 2. According to the results, O<sub>3</sub> was effective in reducing the initial bacterial load to less than 1 log cfu/g for both mesophilic and psychrotrophic bacteria. At 5 and 14 days storage, the use of intermediate immersion time (3 min) was more effective than the short (1 min) or long (5 min) possibly because more time allows the O<sub>3</sub> react with other components



**Figure 2.** Ethylene emission of 'Thomas' sliced tomato washed in water or in ozonated water (0.4 mg/L) during 5 min and stored 14 days at 5 °C. Means ( $n=5$ )  $\pm$ SE.

of fruit tissue undermining its antimicrobial action (Alexandre et al., 2011; Khadre et al., 2001; Restaino et al., 1995).

In the case of yeast, longer ozonated water immersion (3 and 5 min) treatments were more effective in controlling the growth (Table 1). After 14 days of storage, these treatments reached values of about 1 log unit less than control and the immersion for 1 min. No differences were found on mold growth, all treatments showing a similar mold level of  $<1.7$  log ufc/g at day 0 and of  $2.1 \pm 0.1$  log ufc/g at day 14 (data not shown).

Despite the differences, slices from all treatments, washed in water or ozone, maintained acceptable microbial quality since the counts do not exceed the limits established by the Spanish fresh-cut legislation (RD 3484/2000, 2001).

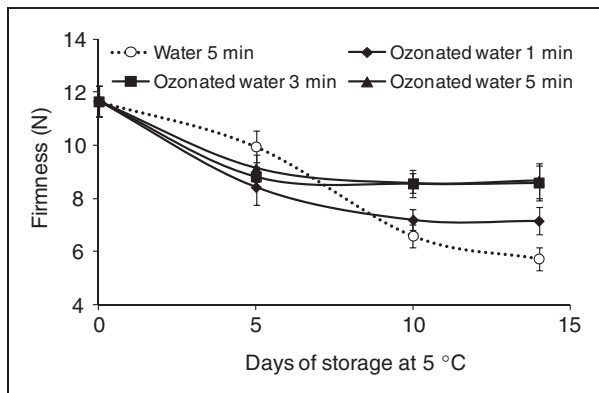
The effect of ozonated water disinfection has been studied for several food products and microorganisms with results of different magnitudes depending on factors like product type, O<sub>3</sub> dose and exposure time. Inactivation of microorganisms by O<sub>3</sub> has been attributed to direct molecular action on the microbes as well as on the radical reactive species generated by the decomposition of ozone molecules (Staehelin and Hoigne, 1985; Williams et al., 2004). Zhang et al. (2005) reported a decrease of 1.7 log units of total microbial counts in fresh-cut celery treated with ozonated water (at 0.18 mg/L). Beltrán et al. (2005) used also ozonated water to reduce total mesophilic population on fresh-cut iceberg lettuce achieving counts of about 1.8 log units less than those registered on control samples, after 13 days of storage at 4 °C. In addition, Akbas and Ölmez (2007) mentioned reduction of

**Table 2.** Microbial growth ( $\log_{10}$  cfu/g) of 'Thomas' sliced tomato washed in water (5 min) or in ozonated water (0.4 mg/L) during 1, 3 or 5 min and stored 14 days at 5 °C. Means ( $n=5$ )  $\pm$ SE

Treatments			Mesophilic	Psychrotrophic	Yeast
Day 0	Water	5 min	$1.7 \pm 0.1$	$1.7 \pm 0.1$	$<1.7$
	Water + O <sub>3</sub>	1 min	$<1$	$<1$	$<1.7$
		3 min	$<1$	$<1$	$<1.7$
		5 min	$<1$	$<1$	$<1.7$
Day 5	Water	5 min	$2.3 \pm 0.1$	$2.0 \pm 0.1$	$<1.7$
	Water + O <sub>3</sub>	1 min	$2.9 \pm 0.2$	$3.1 \pm 0.2$	$<1.7$
		3 min	$1.8 \pm 0.1$	$1.8 \pm 0.2$	$<1.7$
		5 min	$2.3 \pm 0.1$	$2.3 \pm 0.2$	$<1.7$
Day 14	Water	5 min	$3.9 \pm 0.2$	$4.2 \pm 0.4$	$3.0 \pm 0.2$
	Water + O <sub>3</sub>	1 min	$4.1 \pm 0.2$	$4.1 \pm 0.2$	$3.4 \pm 0.2$
		3 min	$3.3 \pm 0.2$	$3.9 \pm 0.1$	$2.1 \pm 0.1$
		5 min	$3.9 \pm 0.1$	$4.8 \pm 0.1$	$<2$

<sup>2</sup>Microbial counts at day 10 are not shown. Mould growth was  $<1.7$  log cfu/g at days 0, 5 and 10. At day 14, all treatments showed similar growth ( $2.1 \pm 0.1$  log cfu/g). Values are means ( $n=3$ )  $\pm$ S.E.

1.7 and 1.5 log units, in mesophilic and psychrotrophic bacteria, respectively, using a dose of 4 mg/L during 2 min. More recently, Alexandre et al. (2011) tested the effectiveness of dipping during 1, 2 and 3 min on ozonated water (0.3 and 2 mg/L) for the control of *Listeria innocua*, artificially inoculated on peppers, total mesophiles on strawberries and total coliforms on watercress, achieving higher reduction ( $2.8 \pm 0.5$ ,  $2.3 \pm 0.4$  and  $1.7 \pm 0.4$  log cycles, respectively) when the highest concentration and highest treatment time (3 min) were used. These previous results confirm the effectiveness of ozonated water reducing microbial load as we have also observed. It is also important to note that the duration of treatment, in particular, long treatments has a major impact on the microbiological quality of the product as we have tested.



**Figure 3.** Firmness of ‘Thomas’ sliced tomato washed in water (5 min) or in ozonated water (0.4 mg/L) during 1, 3 or 5 min and stored 14 days at 5 °C. Means ( $n=5$ )  $\pm$ SE. Analysis of variance showed as significant factors the time of storage and the interactions (time  $\times$  kind of washing). Least significant difference (LSDs) (5%) of time  $\times$  kind of washing = 2.30.

### Firmness

The flesh firmness decreased significantly, between 23 and 28% (depending on the water treatment), in just 5 days of storage (Figure 3). From this moment, the slices treated with ozonated water, regardless of washing length of treatment, showed stable firmness values without significant changes until the end of storage period, while the control suffer a reduction with extension of storage time. At the end of the storage, the ozonated washed slices were statistically different from the control with softening values of 26% when slices were washed for 3 and 5 min, 38% for those washed during 1 min and 50% to the control (related to the initial values). Contrary to this experiment, no effect of the O<sub>3</sub> gas on the firmness maintenance of tomato slices were observed by Aguayo et al. (2006). More recently, Selma et al. (2007) did not find firmness differences between untreated controls and melon treated with gaseous O<sub>3</sub> supplied by carbon monoxide gas (10,000 mg/L during 30 min). However, Alexandre et al. (2012) reported that strawberries treated with ozonated water (0.3 mg/L, time not specified) and stored during 14 days at 4 °C were firmer than those washed in water or the untreated ones.

### Titration acidity, pH and acids contents

A slight increase in the titrable acidity (TA) values, from 0.35 to 0.38 g citric/100 mL, accompanied by a decrease in pH through the storage period was obtained (Table 3). Those changes were observed on day 5, remaining unchanged until the end of storage. Some researchers as Nguyen-the and Carlin (1994) have attributed the slight increase in TA to growth of lactic acid bacteria although in this experiment this bacterial group was not measured. No significant differences between disinfection treatments were observed.

The main organic acids quantified here were citric, malic, succinic, ascorbic and fumaric acid.

**Table 3.** TA, pH, organic acid, TSS, monosaccharides and luminosity (L\*) of ‘Thomas’ sliced tomato washed in water (5 min) or in ozonated water (0.4 mg/L) during 1, 3 or 5 min and stored 14 days at 5 °C. Analysis of variance ( $p \leq 0.05$ ) showed as significant factor the time of storage. Letters differentiate values compared by the least significant difference (LSD) multiple range

Days	TA	pH	TSS	Glucose	Fructose	Ascorbic acid	Fumaric acid	Succinic acid	L*
0	0.35 b	4.30 a	5.7 a	18.92 a	19.22 a	19.70 a	1.01 a	0.03 a	57.1 a
5	0.39 a	4.27 ab	5.1 b	18.30 b	18.79 a	17.18 ab	0.95 a	0.02 b	56.5 a
10	0.38 a	4.26 b	5.1 b	17.43 bc	17.79 b	15.86 b	0.90 b	0.02 b	55.9 a
14	0.38 a	4.26 b	5.2 b	17.13 c	17.83 b	15.30 b	0.93 ab	0.02 b	52.7 b

TA: total acidity (g citric/100 mL); TSS: total solid soluble (g/100 mL); glucose and fructose (g/L); ascorbic and fumaric acid (mg 100/mL); succinic acid (g/100 mL); L\*: luminosity.

According to Souci et al. (1986), citric and malic acid concentration in tomato is around 0.44 and 0.037 g/100 g, respectively, while ascorbic and fumaric acids reach values of about 24.4 and 1.2 mg/100 g, respectively. Our values of citric (0.40 to 0.43 g/100 g), malic (0.04 to 0.06 g/100 g) and fumaric (0.90 to 1.01 mg/100 g) acids were similar to those reported by Souci et al. (1986). However, the ascorbic acid values were lower (Table 3), probably due to the fact that this acid is highly susceptible to oxidative deterioration under acute abiotic stress, a process accentuated by minimum processing as (Chauhan et al., 2011).

None of the factors studied (time of storage and disinfection treatment) influenced the citric and malic acid contents (Table 1). Instead, ascorbic, fumaric and succinic acids decreased only with storage time (Table 3). However, other researchers have found an influence of the ozone treatment in organic acids levels, in particular, in the ascorbic acid. For example, Aguayo et al. (2006) reported a preservation of ascorbic and fumaric acid when tomato slices were disinfected with O<sub>3</sub> gas. However, Chauhan et al. (2011) obtained a reduction in the ascorbic acid contents in sliced carrots washed in ozone (200 mg O<sub>3</sub>/h for 10 min). In fact, the results will change depending on the commodity and dose, duration and ozonation treatment.

### Total soluble solid and sugar contents

Sugars are important components responsible for tomato quality. In our experiment, the initial total soluble solid (TSS) was 5.7° Brix changing only with time of storage with a reduction of about 8% at day 5 and keeping this level during the rest of the storage (Table 3). Fructose and glucose changed throughout the storage (Table 3) and kind of washing (Table 4). Until 10 days, the relationship between glucose and fructose was among 0.97 to 0.98, indicating a similar consumption of both monosaccharides. However, after 14 days at 5°C, the reduction in glucose content was more intense than in fructose (9.5 and 7.2%, respectively). The O<sub>3</sub> treatments, especially those of longer duration, reduced the consumption of these monosaccharides (Table 4). These results are in agreement to Aguayo et al. (2006) who reported that the tomato slices kept in gaseous O<sub>3</sub> had a higher fructose and glucose content than slices kept in air, which the authors attributed to the lower metabolism. Silveira et al. (2010) also preserved these monosaccharides when applied ozonated water (4 mg/L) during 3 min on fresh-cut 'Galia' melon. Contrarily, Zhang et al. (2005) did not find significant difference between total sugar of fresh-cut celery treated with ozonated water (0.18 mg/L) and that of non-treated.

### Color measurement and sensory evaluation

The parameter L\* of the juice color was maintained for 10 days, decreasing at the end of storage (Table 3). The h° values ranged from 49.4 and 56.1 indicating a red color, and remained unchanged during the storage and with no influence of disinfection treatment. The tomato slices treated with ozonated water for 5 min showed more saturation (chroma) than the control, indicating that O<sub>3</sub> imparted a better color purity (Table 4). According to Alexandre et al. (2012) the use of ozonated water was one of the treatments with better color retention on strawberries stored for 14 days at 4°C.

The sensory parameters were only affected by the storage time. Aroma was the most unstable sensory parameter, followed by overall quality and appearance. In just 5 days, these parameters significantly decreased compared to initial scores (Table 5) and scored under limit of marketability at the end of the storage. In contrast, taste and texture remained above the marketability.

None of the disinfected treatments achieved a shelf-life period of 14 days, due to the reduction of appearance

**Table 4.** Monosaccharides and chroma (C\*) of 'Thomas' sliced tomato washed in water (5 min) or in ozonated water (0.4 mg/L) during 1, 3 or 5 min and stored 14 days at 5°C. Analysis of variance ( $p \leq 0.05$ ) showed as significant factor the washing treatment. Letters differentiate values compared by the LSD multiple range

Kind of washing	C*	Glucose	Fructose
Water 5 min	20.9 b	17.46 b	17.86 b
Water + O <sub>3</sub> 1 min	21.6 ab	17.92 ab	18.36 ab
Water + O <sub>3</sub> 3 min	21.7 ab	18.12 a	18.57 a
Water + O <sub>3</sub> 5 min	22.3 a	18.29 a	18.85 a

Glucose and fructose (g/L).

**Table 5.** Sensorial parameters (1 to 9) of 'Thomas' sliced tomato washed in water (5 min) or in ozonated water (0.4 mg/L) during 1, 3 or 5 min and stored 14 days at 5°C. Analysis of variance ( $p \leq 0.05$ ) showed as significant factor the time of storage. Letters differentiate values compared by the least significant difference (LSD) multiple range

Days	Appearance	Taste	Aroma	Texture	Overall quality
0	8.0 a	7.7 a	8.0 a	7.7 a	8.0 a
5	7.1 b	7.3 ab	7.3 b	7.3 ab	6.9 b
10	6.3 b	6.6 bc	6.3 c	6.7 bc	6.4 b
14	4.8 c	6.0 c	3.8 d	6.3 c	4.6 c



(4.5 for control and 5.0 for ozonated treatments) and mainly loss of aroma in all treatments (3.7 to 4.0). These low qualifications determined that the overall quality was less than limit of marketability. However, it was possible to achieve a shelf life of 10 days at 5 °C in 'Thomas' tomato slices washed in water or ozonated water.

These results differ from that observed when tomato slices were treated with gaseous O<sub>3</sub>. Aguayo et al. (2006) found that tomato slices maintained a good appearance and overall quality after 15 days of storage at 5 °C while in control fruit these parameters were below the marketability limit. Other researchers have found a positive effect using ozonated treatment to keep or improve sensorial quality. For example, fresh-cut green leaf lettuce treated with ozonated water (2 mg/L) gained significantly better scores in all sensory parameters compared with the chlorine (100 mg/L)-treated samples at day 9 of storage at 4 °C (Ölmez and Kretzschmar, 2009). Silveira et al. (2010) who used ozonated water (0.4 mg/L) to disinfect fresh-cut 'Galia' melon without negative effects on the sensory quality, maintaining the quality at acceptable levels for a shelf-life of 10 days at 5 °C. In our experiment, the changes in sensorial parameters were only influenced by time of storage and it was not possible to obtain a significant response to ozonated water.

## CONCLUSIONS

Treating tomato slices with 0.4 mg/L ozonated water for 3 min achieved the best firmness retention and the microbial quality keeping the glucose and fructose levels. Extending the duration of ozonated treatment to 5 min did not improve the microbiological quality.

## FUNDING

The authors are grateful to the Spanish Ministry for Education and Science (project AGL 2007-63861/ALI) for financial support.

## REFERENCES

Aguayo E, Escalona VH and Artés F. (2006). Effect of cyclic exposure to ozone gas on physicochemical, sensorial and microbial quality of whole and sliced tomatoes. *Postharvest Biology and Technology* 39: 169–177.

Akbas MY and Ölmez H. (2007). Effectiveness of organic acids, ozonated water and chlorine dippings on microbial reduction and storage quality of fresh-cut iceberg lettuce. *Journal of Science Food and Agriculture* 87: 2609–2616.

Alexandre E, Brandão T and Silva C. (2012). Efficacy of non-thermal technologies and sanitizer solutions on microbial load reduction and quality retention of strawberries. *Journal Food Engineering* 108: 417–426.

Alexandre E, Santos-Pedro D, Brandão T and Silva C. (2011). Influence of aqueous ozone, blanching and combined treatments on microbial load of red bell peppers, strawberries and watercress. *Journal Food Engineering* 105: 277–282.

APHA, American Public Health Association (1998). *Standard methods for the examination of water and wastewater*. Washington, DC: The American Public Health Association.

Artés-Hernández F, Aguayo E, Artés F and Tomás-Barberán F. (2007). Enriched ozone atmosphere enhances bioactive phenolic in seedless table grapes after prolonged shelf life. *Journal Science Food and Agriculture* 87: 824–831.

Beltrán D, Selma MV, Marín A and Gil MI. (2005). Ozonated water extends the shelf life of fresh-cut lettuce. *Journal Agriculture and Food Chemistry* 53: 5654–5663.

Bramley PM. (2000). Is lycopene beneficial to human health? *Phytochemistry* 54: 233–236.

Chauhan OP, Raju PS, Ravi N, Singh A and Bawa AS. (2011). Effectiveness of ozone in combination with controlled atmosphere on quality characteristics including lignification of carrot sticks. *Journal Food Engineering* 102: 43–48.

Delaquis PJ, Fukumoto LR, Toivonen PMA and Cliff MA. (2004). Implications of wash water chlorination and temperature for the microbiological and sensory properties of fresh-cut iceberg lettuce. *Postharvest Biology and Technology* 31: 81–91.

Güzel-Seydim ZB, Greene AK and Seydim AC. (2004). Use of ozone in the food industry. *Lebensmittel Wissenschaft und Technologie* 37: 453–460.

Graham DM. (1997). Use of ozone for food processing. *Food Technology* 51: 72–75.

Khadre MA, Yousef AE and Kim JG. (2001). Microbiological aspects of ozone applications in food: A review. *Journal of Food Science* 66: 1242–1252.

Kim JG, Yousef A and Chism G. (1999). Use of ozone to inactivate microorganism on lettuce. *Journal Food Safety* 19: 17–34.

Koseki S and Isobe S. (2006). Effect of ozonated water treatment on microbial control and on browning of iceberg lettuce (*Lactuca sativa* L.). *Journal of Food Protection* 69: 154–160.

Martín-Belloso O, Soliva-Fortuny R and Oms-Oliu G. (2006). Fresh-cut fruits. In: Hui YH (ed.) *Handbook of Fruits and Fruit Processing*. Oxford: Blackwell Publishing, pp. 129–144.

Martínez-Sánchez A, Allende A, Cortes-Galera Y and Gil MI. (2007). Respiration rate response of four baby leaf Brassica species to cutting at harvest and fresh-cut washing. *Postharvest Biology and Technology* 47: 382–388.

Nguyen-the C and Carlin F. (1994). The microbiology of minimally processed fresh fruits and vegetables. *Critical Reviews in Food Science and Nutrition* 34: 371–401.

Ölmez H and Kretzschmar U. (2009). Potential alternative disinfection methods for organic fresh-cut industry for minimizing water consumption and environmental impact. *Lebensmittel Wissenschaft und Technologie* 42: 686–693.

- RD 3484/2000 (2001). *Boletín Oficial del Estado*. Spain vol 11, pp.1435–1441.
- Rico D, Martín-Diana A, Frías J, Henehan G and Barry-Ryan C. (2006). Effect of ozone and calcium lactate treatments on browning and texture properties of fresh-cut lettuce. *Science Food and Agriculture* 86: 2179–2188.
- Restaino L, Frampton EW, Hemphill JB and Palikar P. (1995). Efficacy of ozonated water against various food-related microorganisms. *Applied Environmental Microbiology* 61: 3471–3475.
- Skog L and Chu C. (2000). Ozone technology for shelf life extension of fruits and vegetables. *Acta Horticulturae* 553: 285–291.
- Selma M, Beltrán D, Allende A and Gil MI. (2007). Elimination by ozone of *Shigella sonnei* in shredded lettuce and water. *Food Microbiology* 24: 492–499.
- Silveira AC, Aguayo E and Artés F. (2010). Emerging sanitizers and Clean Room packaging for improving the microbial quality of fresh-cut ‘Galia’ melon. *Food Control* 21(6): 863–871.
- Souci SW, Fachmann W and Kraut H (1986). *Food Composition and Nutrition Tables*. 3rd edn. München: Deutsche Forschungsanstalt für Lebensmittelchemie, Garching b.
- Staehelin J and Hoigne J. (1985). Decomposition of ozone in water in the presence of organic solutes acting as promoters and inhibitors of radical chain reactions. *Environmental Science and Technology* 19: 1206–1213.
- Williams RC, Sumner SS and Golden DA. (2004). Survival of *Escherichia coli* O157:H7 and Salmonella in apple cider and orange juice as affected by ozone and treatment temperature. *Journal of Food Protection* 67(11): 2381–2386.
- Zhang L, Lu Z, Yu Z and Gao X. (2005). Preservation of fresh-cut-celery by treatment of ozonated water. *Food Control* 16: 279–283.