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Disinfection of selected vegetables under nonthermal treatments: Chlorine, acid citric, ultraviolet light and ozone

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ABSTRACT

Lettuce, tomatoes and carrots were evaluated under four disinfection methods. Chlorine (50, 100 and 200 ppm) was compared for effectiveness with citric acid (0.5, 1 and 1.5%), ultraviolet light (UV-C) (0.65 and 1.6 mW/cm^2) and ozone (5 ppm) to inactivate *Escherichia coli* ATCC 11775. Processing times were from 3 min up to 60 min. Hunter color parameters, color functions (ΔE, *hue, chroma*), tomato color index (TCI) and whiteness index (WI) were evaluated after disinfection. Results showed that citric acid was not effective for inactivation of *E. coli* at the tested conditions. UV-C was effective in the inactivation of the microorganism when fluence was higher, being more effective in the smooth surface of tomato (2.7 log). Meanwhile, ozone was also able to inactivate bacteria in tomatoes (2.2 log) after only 3 min. Carrots and lettuce showed lower inactivation for all treatments because of their porous and roughened surfaces. UV-C was the treatment that most affected the color of the produce; it generated browning of lettuce, and increase of TCI and WI of carrots. Ozone also affected the greenness of lettuce. Concentration, dose and processing times of novel disinfection methods need to be evaluated not only for microbial counts, but also sensory properties.

1. Introduction

The economic cost related with foodborne illnesses in the United States is higher than 50 billion of dollars per year involving more than 48 millions of persons (Scharff, 2012). Some of the pathogenic microorganisms found in fresh produce and associated with these foodborne outbreaks are *Escherichia coli, Salmonella, Shigella* spp. and *Listeria monocytogenes* (Park, Alexander, Taylor, Costa, & Kang, 2008). Several recalls in the United States have been issued in 2011 involving the presence of *E. coli* O157:H7 in romaine lettuce and *Salmonella* in grape tomatoes and carrots, sold as separate units or as a part of pre-packaged salads, involving the entire country (FDA, 2012). *E. coli* has shown the ability to attach very strongly to leafy structures, which makes it difficult to remove the cells from fresh produce (López-Gálvez, Allende, Selma, & Gil, 2009).

Some chemicals have been evaluated for use as disinfectant agents in produce, such as chlorinated water and chlorine dioxide (Park et al., 2008). However, the use of chlorine has been associated with the formation of carcinogenic compounds in the last few years, and some pathogens have been shown to be more resistant to the lethal action of these compounds (Allende, Selma, López-Gálvez, Villaescusa, & Gil, 2008). There is a current need to provide fresh and microbiologically safe fresh-cut produce for consumers (Allende, Tomás-Barberán, & Gil, 2006; Park et al., 2008). Also, there has been an important increase in the sale of fresh produce in the last several years because of consumers' trend to eat healthy food (Thilmany et al., 2007). Thus, new sanitizers or technologies to disinfect fruits and vegetables should be efficient in the inactivation of pathogens while maintaining the sensory quality of the product (Allende et al., 2008).

Some of the novel technologies of disinfection methods in food include the use of ultraviolet light, ultrasound, ozone, irradiation, cold plasma, and organic acids, among others. Ultraviolet light-C ($100 < \lambda < 280$ nm) is able to inactivate microorganisms because of the irreversible damage to DNA (Otto et al., 2011). It has been used to disinfect water, air, surfaces, containers and vegetable commodities (Allende et al., 2006). Ozone has been approved in the United States to be used in gaseous or liquid phase as a disinfectant of food because it has better antimicrobial properties than chlorine (Kim, Yousef, & Khadre, 2003). Ozone is commonly used to disinfect drinking water; however, one of the main challenges of ozone as a sanitizer is the poor stability when organic matter is present (Selma, Allende, López-Gálvez, Conesa, & Gil, 2008). Meanwhile,

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organic acids are used in the food industry to extend the shelf-life of some products, but when used in higher concentrations these acids are able to inactivate microorganisms because of the acidification of the cytoplasm of the bacterial cell (Virto, Sanz, Álvarez, Condón, & Raso, 2005). Some of the organic compounds used for disinfection are propionic, acetic, malic, citric, lactic, and tartaric acid, among others (Huang & Chen, 2011; Rahman, Jin, & Oh, 2010; Sagong et al., 2011).

The aim of this work was to evaluate the use of three nonthermal treatments in the inactivation of a surrogate microorganism of the pathogenic *E. coli* O157:H7 in fresh produce and to compare them with the conventional chlorination process. Cells of the non-pathogenic *E. coli* ATCC 11775 were used and inoculated in three challenge surfaces: carrots, tomatoes and lettuce and were exposed to ultraviolet light, ozone and citric acid solutions during certain disinfection times.

2. Materials and methods

2.1. Vegetable samples

Three different samples were chosen to study the degree of disinfection: a green leafy product such as romaine lettuce (*Lactuca sativa* L. var. *longifolia)*, a smoothed surface fruit such as grape tomatoes (*Lycopersicon lycopersicum)* and a porous surface vegetable such as baby carrots (*Daucus carota* L.). All of the vegetables were purchased in a local supermarket and kept under refrigerated conditions (4 °C) until used. Initial mesophilic loads were evaluated for each vegetable.

2.2. E. coli ATCC 11775

2.2.1. Growth

A strain of *E. coli* ATCC 11775 was used as a surrogate microorganism of *E. coli* O157:H7 because of its higher resistance (Gurtler, Rivera, Zhang, & Geveke, 2010). *E. coli* ATCC 11775 was rehydrated with 5 ml of sterile nutrient broth (Bacto: Becton, Dickinson and Co., Sparks, MD). After 30 min the cell suspension was inoculated into 100 ml of sterile nutrient broth and incubated at 35 °C with continuous agitation at 225 rpm in an orbital shaker. Absorbance was read at 564 nm in a 8452A diode array spectrophotometer (Hewlett-Packard, Palo Alto, CA) until reaching the early stationery phase, approximately after 17 h. Bacterial growth was assessed using pour-plate enumeration for *E. coli* cells. One ml of the early stationery phase plus one ml of a glycerol solution (20 ml glycerol/100 ml sterile water) were mixed together and stored at −21 °C.

2.2.2. Inoculation of vegetables

For inoculation of vegetables, samples were prepared as follows. All vegetables were rinsed with sterile water to remove some of the natural flora (only for *E. coli* experiments) and any other matter (e.g. soil) before treatment. For lettuce, two to three outer leaves were discarded and the internal leaves were cut with a sterile knife into small pieces of approximately 5 × 5 cm with a weight of about 1 g (±0.15 g). Carrots were cut in small discs with another sterile knife (approximately 1 g ± 0.23 g). Both vegetables needed to be cut to fit the treatment chamber of the ozone equipment. Grape tomatoes were left whole (9.48 g ± 1.01). For infection of the produce, the stock culture was added to 100 ml of sterile nutrient broth, agitated by hand for few seconds and then placed in an orbital shaker at 37 °C (218 rpm) for 17 h. The next day, 0.5 ml of the culture were added to 500 ml of sterile water and vegetable samples were left in direct contact with the microbial solution (10^7 cfu/ml) for 30 min. After that, samples were dried in a laminar hood for 30 min to fix the bacteria on the surface of the product; this time allowed to have a high initial microbial load and was in agreement with the reported by Lang, Harris, and Beuchat (2004); Yaun, Sumner, Eifert, and Marcy (2004) and Sapers and Jones (2006). Afterward, samples were transferred aseptically to the different solutions or treatments to start the disinfection process. Several samples of each vegetable after the exposition with *E. coli* cells were taken directly to evaluate the time zero for each sample.

2.2.3. Microbiological analysis

Approximately 1 g of each vegetable (for lettuce and carrots) and a whole tomato were placed into 9 ml and 90 ml of sterile peptone water (0.1%), respectively, inside a sterile plastic bag and homogenized with a Seward 400 Circulator Stomacher (Seward, Ltd., London, U.K.). Serial dilutions were made with sterile peptone from the different disinfection solutions or treatments, and also for the innoculum solution. For the initial loads of mesophiles on vegetables, samples were pour-plated into plate count agar (Difco, Becton, Dickinson and Co., Sparks, MD). Dishes were incubated at 35 °C for 48 h and then mesophiles were counted. For *E. coli* dilutions were pour-plated on EMB Agar (Neogen©, Lansing, MI). Dishes were incubated at 35 °C for 48 h and then bacteria were counted.

2.3. Disinfection treatments

2.3.1. Ultraviolet light

The ultraviolet treatment was applied in a cabinet equipped with a Sylvania germicidal lamp, G30 T8 RG3 30W Hg (Japan). The ultraviolet source has a maximum radiation peak at 253.7 nm. Experiments were conducted at room temperature (24 °C). Two working distances were used, 31 and 70 cm; vegetables were placed at these distances from the UV irradiated lamp. Samples were placed on sterile plastic petri dishes. Fluence was calculated as described by Bolton and Linden (2003) having two values, 1.6 mW/cm^2 and 0.65 mW/cm^2, for the shortest and longest distances, respectively. Processing times were 0, 3, 6, 9, 12, 15, 30 and 60 min. At the end of the longest exposure time, temperature was 25 (±0.5) °C. After treatment, samples were aseptically collected in sterile plastic bags with 0.1% peptone solution and processed for microbiological counts.

2.3.2. Ozone

Ozone was generated with an air cooled corona discharge equipment model lab 11 (Pacific Ozone, Egret Court Benicia, CA) connected to a packed bed reactor (7.3 cm internal diameter) with an approximately 0.1 cm bed depth. The batch reactor is made of 316 stainless steel (SS) and the connectors are made of 304 SS, 316 SS or PFTE. A thermocouple type K (Omega Engineering, Inc. Stamford, CT, USA) was installed inside the chamber to record the temperature profile during experiments to ensure a nonthermal treatment. A concentration of 5 ppm of ozone was used for the experiments, using a voltage of 3.4 V, flow rate of 2 standard lpm and pressure of 6 psi. Samples were placed on the bed during 3, 6, 9, 12 and 15 min. At the exit, the ozone concentration of the chamber was confirmed with a precision gas mass flow meter (Apex, Canton, GA). Equipment was calibrated with an iodine wet chemistry method to quantify ozone concentration (Rakness, Henry, & Langlais, 2000).

2.3.3. Chlorine solutions

Three different concentrations of sodium hypochlorite were prepared from a solution (6% v/v) of ultra germicidal bleach (Food Services America, Inc., Seattle, WA). Sterile water was mixed with sodium hypochlorite to have a final concentration of 50, 100 and 200 ppm (500 ml each). Chlorine concentration was verified with LaMotte® chlorine test papers (Chestertown, MD). The pH of the chlorine solution was adjusted to 6.5 in accordance with CFSAN

(1998). Vegetables were exposed to this agent during 0, 3, 6, 9, 12 and 15 min. After each time, samples were collected and transferred to sterile plastic bags with 0.1% peptone solution and processed for microbiological counts.

2.3.4. Citric acid solutions

Three different concentrations of citric acid were tested against *E. coli* cells. Granular citric acid monohydrate (Sigma, St. Louis, MO) was mixed with sterilized water to have a final concentration of 0.5%, 1.0% and 1.5% (w/v) in a final volume of 500 ml for each solution. Exposition time of the vegetables to citric acid was 0, 3, 6, 9, 12 and 15 min. Experiments were conducted at room temperature (21 °C). After that, samples were collected and transferred to sterile plastic bags with 0.1% peptone for further analysis.

2.4. Color

Color parameters were measured in the three vegetables before and after processing. A Minolta CM-2002 spectrophotometer (Minolta Camera, Co., Osaka, Japan) was used in the reflection mode. Lightness to darkness (L^*), redness to greenness (a^*) and yellowness to blueness (b^*) were quantified in all of the vegetables in at least four different points of the product.

The net color difference (ΔE^*) was determined using L^*, a^* and b^* values, comparing them with the values of unprocessed samples:

$$\Delta E^* = \sqrt{(\Delta L^*)^2+(\Delta a^*)^2+(\Delta b^*)^2} \quad (1)$$

Hue angle (h^*) was determined using the following relationship:

$$h^* = \tan^{-1}\left(\frac{b^*}{a^*}\right) \quad (2)$$

and the chroma or saturation index (C^*) was evaluated using this equation:

$$C^* = \left(a^{*2} + b^{*2}\right)^{1/2} \quad (3)$$

The tomato color index (TCI) was calculated as described by Obande, Tucker, and Shama (2011) and Clément, Dorais, and Vernon (2008) with the following equation:

$$\mathrm{TCI} = \frac{2000{*}a^*}{\sqrt{L^{**}(a^{*2}+b^{*2})}} \quad (4)$$

Also, the whiteness index (WI) was calculated for lettuce and carrots as follows:

$$\mathrm{WI} = 100 - \left[\left(100 - L^*\right)^2 + a^{*2} + b^{*2}\right]^{0.5} \quad (5)$$

2.5. Electron microscopy

Some samples were analyzed with electron microscopy after processing with the disinfectant agents. A small piece of each sample was mounted on aluminum stubs and observed directly (no sample preparation was required) in the Quanta 200F Environmental Scanning Electron Microscope (FEI, Field Emission Instruments, Hillsboro, OR) by using the environmental mode at 10 and 20 kV.

2.6. Statistical analysis

All experiments were carried out at least in duplicate. During each experiment at least five samples were taken from each vegetable to conduct microbial counts and color readings. Statistical analysis of the results was done using Microsoft Excel. Analysis of variance was conducted using an SAS program with a significant level of α 0.05. A pair wise Tukey's test was used to find significant difference between treatments using α 0.05.

3. Results and discussion

3.1. Chlorine solutions

In Fig. 1, comparison in the degree of disinfection with chlorine solutions at different concentration is shown for three vegetables. First, in Fig. 1a disinfection is shown for carrots. The main challenge in this vegetable is the porous surface that allows bacteria to migrate to the inner tissue and thus be protected from the sanitizer agent. In this figure, disinfection varies in accordance with the concentration of chlorine and exposure time, reaching a final inactivation of 3.5 log after 15 min Klaiber, Baur, Wolf, Hammes, and Carle (2005) found a reduction of mesophiles in carrots of about 1.7 log when chlorinated cold water (200 ppm, 4 °C) was used, and inactivation was enhanced (2.3 log) when the chlorinated water was warmed up (50 °C) during 120 s. Meanwhile, Ruiz-Cruz, Acedo-Felix, Díaz-Cinco, Islas-Osuna, and González-Aguilar (2007) found about 2.5 log reduction of *E. coli* O157:H7 in carrots when 200 ppm was used in tap water during 2 min; recirculation of water did not have a positive effect on the inactivation.

Fig. 1b shows disinfection in lettuce; it is well known that in leafy vegetables bacteria migrates easily to some points of difficult access for the sanitizers and protects the microorganisms. The highest inactivation for romaine lettuce was 3.5 log reduction and was achieved after 15 min in contact with the solution of 100 ppm. In another study with iceberg lettuce, the vegetable was washed for 1 min with a chlorine solution of 100 ppm and inactivation of coliforms was 1.4 log (Allende et al., 2008). Chlorinated water is by far the most used disinfectant method for washing produce; however, its effectiveness in sanitizing the vegetables is minimal, with less than 2–3 log reduction (Chang & Schneider, 2012; Park et al., 2008).

In a detailed study with lettuce and chlorine (200 ppm), it was observed that cells of *E. coli* survived to a minor degree in the surface of the leaves rather than in the inner structures of a vegetable such as stomata or damage tissue. Cells were able to protect themselves from the action of chlorine in some areas of difficult access for the sanitizer; when bacteria penetrated 0–10 μm, the viability was between 50.8 and 45.6%. Meanwhile when the penetration was 30–40 μm viable cells were 68.3% (Takeuchi & Frank, 2001).

Finally, Fig. 1c shows the degree of disinfection using chlorine in tomatoes. In this case, the result is probably due to the smooth surface of the tomato, the inactivation of *E. coli* after 15 min and using 200 ppm of chlorine was complete (8.06 log) and non-viable cells were observed in the range of the detection limit (25–250 cfu/ml) either in the scar or in the tomato surface. Sapers and Jones (2006) found similar results; and because the inoculation time was short, there might not have been enough time for *E. coli* cells to internalize into the tomato core through the scar. In a study with *Salmonella* cells inoculated in tomatoes, sodium hypochlorite was able to inactivate up to 5.5 log after 60 s using an overhead spray and brush roller system, compared to only 3.3 log at the same concentration and time, but using a simulated flume (Chang & Schneider, 2012).

3.2. Citric acid

Using citric acid as a sanitizer did not show important results in the present research under the tested conditions. For carrots and

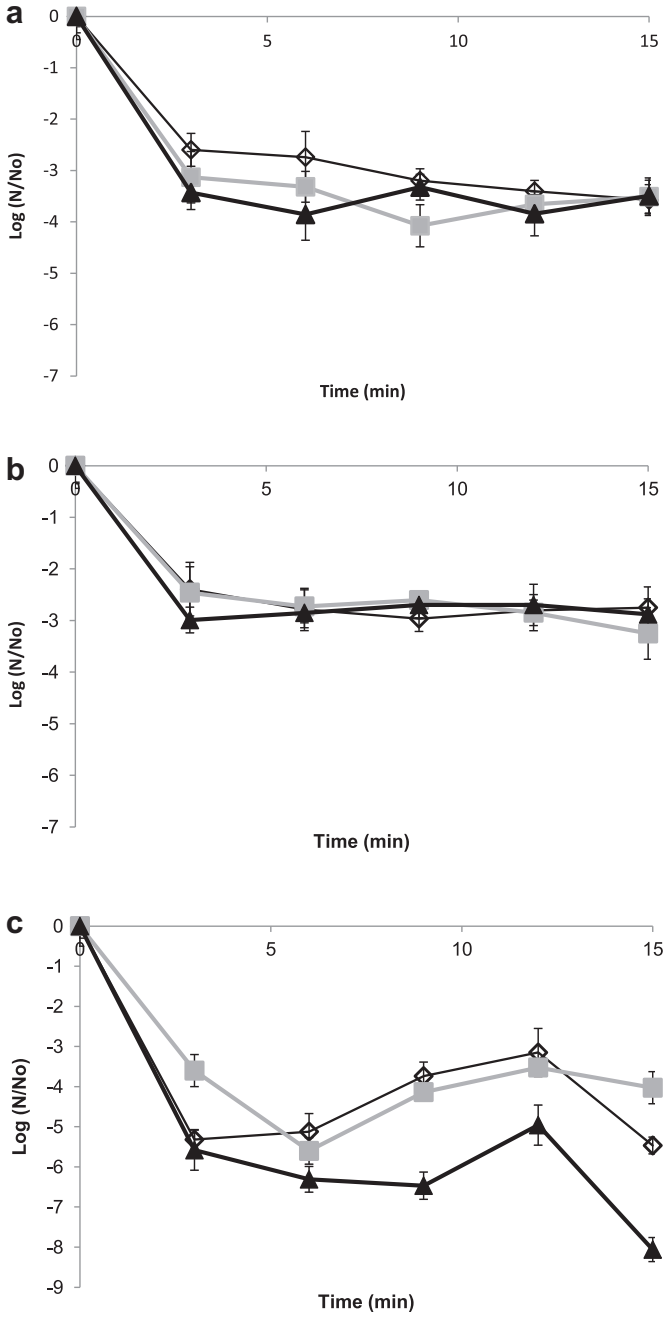

Fig. 1. Inactivation of *E. coli* in a) carrots; b) lettuce; and c) tomatoes using chlorine solutions at three different concentrations: (◇) 50 ppm, (▩) 100 ppm, and (▲) 200 ppm. Each experiment was run at least in duplicate and at least five samples were analyzed in each experiment. Initial count was 10^7–10^8 cfu/ml.

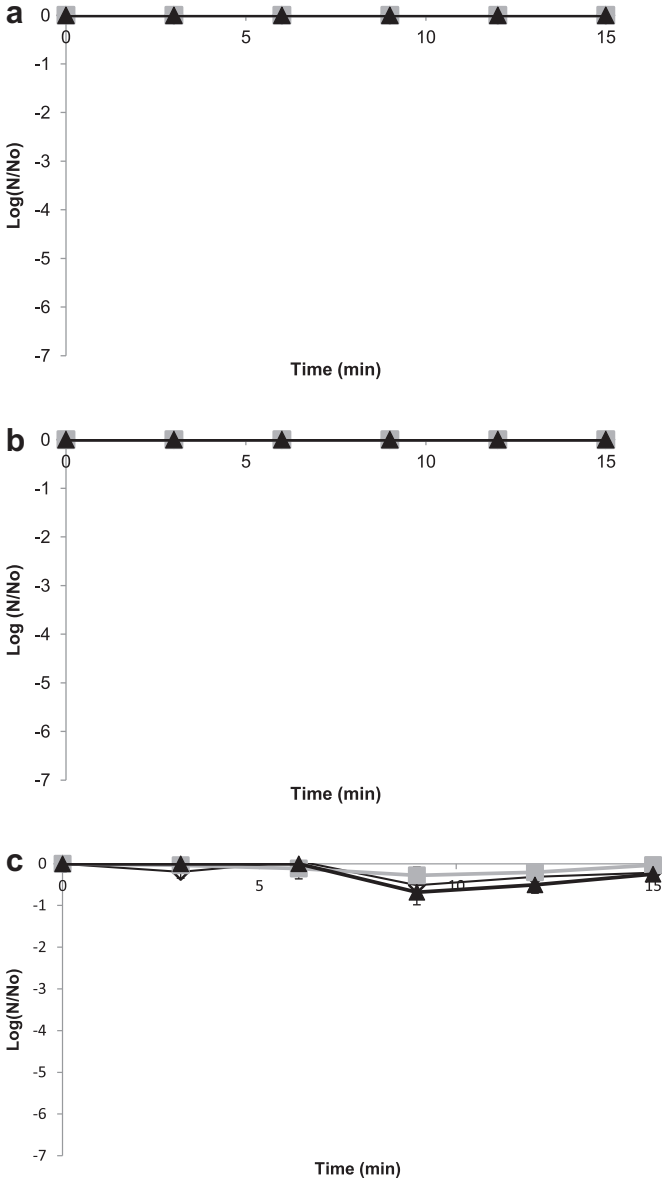

Fig. 2. Inactivation of *E. coli* in a) carrots; b) lettuce; and c) tomatoes using citric acid at three different concentrations: (◇) 0.5%, (▩) 1.5%, (▲) 3%. Each experiment was run at least in duplicate and at least five samples were analyzed in each experiment. Initial count was 10^7–10^8 cfu/ml.

lettuce no inactivation was reported after 15 min regardless of the concentration (Fig. 2a and b). Meanwhile, in the case of tomato, a very low inactivation (0.7 log) was observed after 9 min using the highest concentration (3%). In a study conducted with *Yersinia enterocolitica* using different concentrations of citric acid, 3% did not show any inactivation when the solution was used at 20 °C even after 30 min, but inactivation was enhanced when temperature was raised up to 40 °C and when higher concentration of citric acid was used (5–20%) (Virto et al., 2005). When citric acid (6 g/L) was used to inactivate *E. coli* O157:H7 in fresh-cut cilantro, less than 1 log-reduction was achieved (Allende, McEvoy, Tao, & Luo, 2009). Meanwhile, in another study using lettuce, citric acid at 0.5% and 1% at room temperature was able to inactivate 0.74 and 1.15 log of *E. coli* O157:H7 after 5 min of treatment having an initial population of 8.36 log, but also having the presence of *L. monocytogenes* (7.45 log) and *Salmonella* Typhimurium (8.28 log) as competitive flora (Sagong et al., 2011). Contrasting results were found in lettuce using the same cocktail of bacterial strains, having *E. coli* O157:H7 with an initial count of 7.03 log; after 10 min of contact with citric acid (1%, room temperature) the final count was 3.84 (Park et al., 2011).

3.3. Ultraviolet light

In the case of ultraviolet light, results are shown in Fig. 3. Firstly, carrots (Fig. 3a) did not show any inactivation even after 60 min of exposure to radiation. Working distance, related with the fluence,

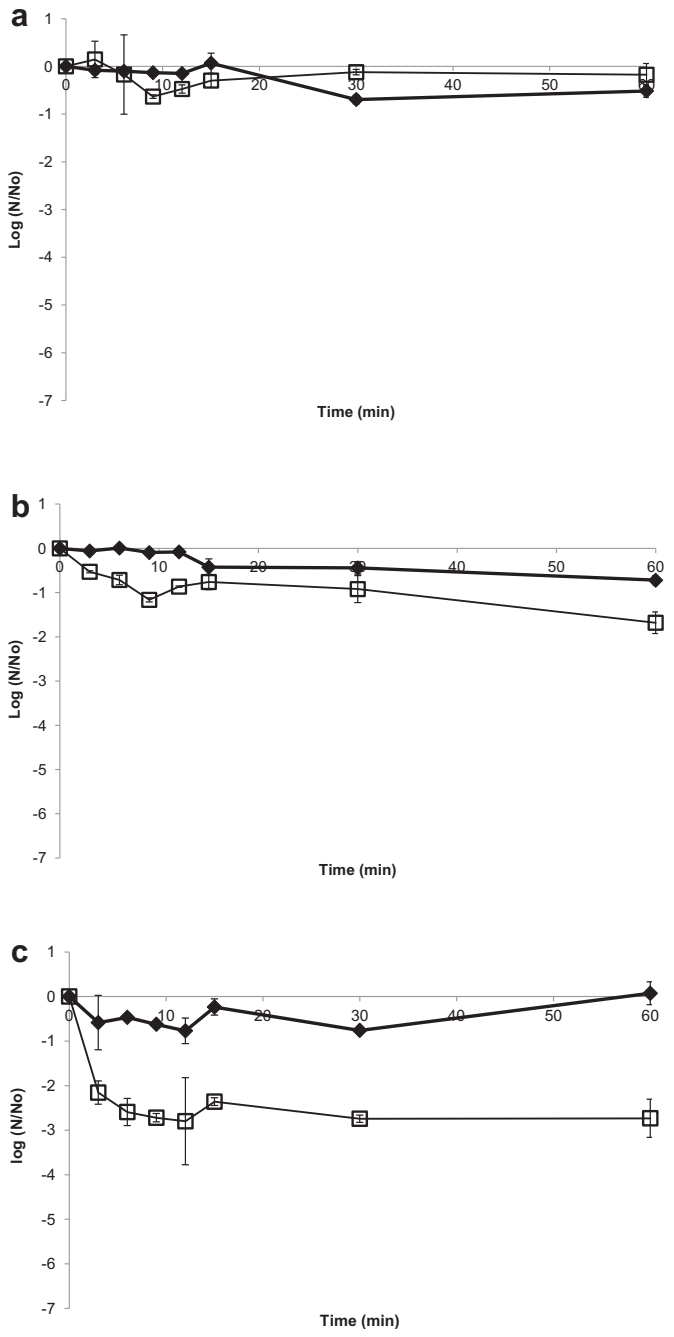

Fig. 3. Inactivation of *E. coli* in a) carrots; b) lettuce; and c) tomatoes using ultraviolet light at two working distances: (◆) 70 cm: total fluence 0.65 mW/cm^2 and (□) 31 cm: total fluence 1.6 mW/cm^2. Each experiment was run at least in duplicate and at least five samples were analyzed in each experiment. Initial count was 10^7–10^8 cfu/ml.

did not affect the cells. In the case of lettuce (Fig. 3b) an inactivation of approximately 1.7 log was achieved after 60 min of treatment. The effect of the working distance is very clear in this case. When samples are far from the UV lamp, inactivation is minimal or null compared to when the samples are closer to the radiation source, allowing better penetration of the UV light into the product and interacting in a higher degree with the microorganisms.

Finally, Fig. 3c shows the degree of inactivation in tomatoes using UV-light. The effect of the smooth surface of tomato is shown again in this example. Inactivation at short working distance and higher fluence (1.6 mW/cm^2) was higher from the very first minutes of treatment. After 60 min of treatment the inactivation was about 2.8 log when samples were closer to the radiation source. These results agree with Otto et al. (2011) which mentions that the efficiency of ultraviolet light is increased when the distance from the radiation source is decreased or when the penetration depth in the product is bigger, such as in transparent liquids. Schnek, Raffellini, Guerrero, Blanco, and Alzamora (2011) showed a reduction of *E. coli* of about 7.2 log after 3 min of treatment (ultraviolet dose of 120 mJ/cm^2) and it was increased up to 8.5 log after 8 min (ultraviolet dose of 330 mJ/cm^2) in peptone water, which demonstrates that the fact of having a clear liquid enhances the penetration of ultraviolet radiation. Studies on tomatoes to inactive molds with ultraviolet light (1.3–40 kJ/m^2) were shown to extend the shelf-life of the product because of the delay on ripening (Yaun et al., 2004). Doses applied in the present work for the shortest working distance were from 2.9 to 57.6 kJ/m^2. In another study, apples, tomatoes and lettuce were disinfected with ultraviolet light (maximum fluence 24 mW/cm^2) and the inactivation of *E. coli* was faster and higher in apples because of the smooth surface, followed by tomatoes and lettuce. However inactivation was not higher than 2.5 log reduction (Yaun et al., 2004).

Microorganisms found protective sites in carrots and lettuce and were able to migrate to these sites when UV radiation was applied. In the case of tomatoes, since this fruit has a smooth surface and the protective sites are limited, microorganisms were completely exposed to the radiation and inactivation was achieved. Gram negative bacteria are also more sensitive to ultraviolet light rather than gram positive bacteria (Otto et al., 2011). The composition of the cell wall in gram negative bacteria does not offer any protection to the cells when they are exposed to different sanitizers, which act directly on the cellular inner structures.

3.4. Ozone

Finally, the disinfection of produce with ozone is shown in Fig. 4. Carrots and lettuce showed a low degree of inactivation even after 15 min. Meanwhile, tomatoes again showed the highest inactivation with 2.2 log reduction after 15 min Singh, Singh, Bhunia, and Stroshine (2002) found that after using 5.2 mg/L of gaseous ozone (15 min) in shredded lettuce and baby carrots, the inactivation of *E. coli* O157:H7 was approximately 1.6 and 2.5 log-reduction, respectively, showing that disinfection of fresh produce sometimes requires the combination of several treatments, such as washing, to achieve higher inactivation. There are reports about the efficiency of ozone in vegetable products that present some injuries and wounds, in which bacteria were located and protected from the action of ozone and were not inactivated (Kim et al., 2003).

In the case of carrots, Selma et al. (2008) found that inactivation of mesophiles and total coliforms in wash water from carrots using ozone (80 mg/min) was about 4 and 1 log-reduction, respectively, after 20 min of treatment. Ozone is responsible for the oxidation of lipids on the cells; it acts on the unsaturated lipids of the cell membrane, and in the lipopolysaccharides coat of gram-negative bacteria, enzymes and genetic material, promoting the death of the microorganism (Kim et al., 2003).

The profile of temperature is shown for this technology (Fig. 4b). During processing, the temperature was kept at room temperature (20 °C) and there was no important increase by the end of the process, confirming that inactivation with ozone is a nonthermal process.

A number of references mention that disinfection of produce with chemical sanitizers or washing reduces the natural flora only in 2–3 log reduction (Gil, Selma, López-Gálvez, & Allende, 2009),

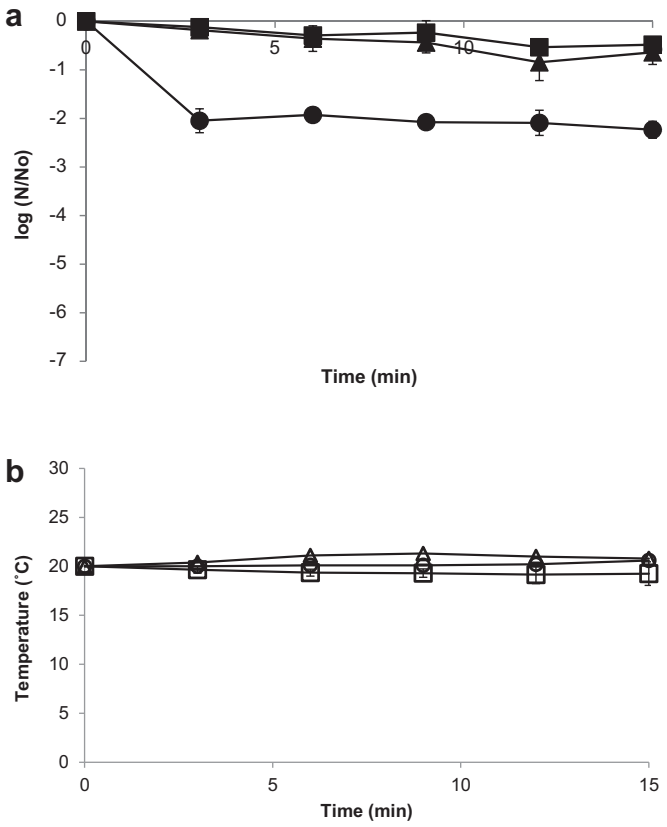

Fig. 4. a) Inactivation of *E. coli* in (▲) carrots, (■) lettuce and (●) tomatoes using ozone at 5 ppm; (b) profile of temperature during disinfection of vegetables with ozone, (△) carrots, (□) lettuce and (○) tomatoes. Each experiment was run at least in duplicate and at least five samples were analyzed in each experiment. Initial count was 10^7–10^8 cfu/ml.

which would not be enough when the concentration of pathogens in the vegetables is higher.

3.5. Color

Changes in color were quantified in all vegetables, fresh and processed with the different treatments, after the longest exposure time. Results are shown in Table 1. Firstly, for lettuce, there were significant changes in the luminosity and redness of almost all the samples. However, the sample treated with ozone was shown to be different ($p < 0.05$) from the rest of the samples. In particular, this sample showed loss in the characteristic green color after processing; the leafy structure of lettuce showed a white coloration after ozone treatment, which when expressed in terms of Hunter's color parameters was an increase of L^* value, as well as in redness and yellowness of the sample, as shown in Table 1. In Fig. 5, the control sample and the sample treated with ozone after 15 min are shown. Samples that showed the highest net change of color (ΔE) were the one treated with UV at highest fluence and the one treated with ozone. However, no important changes were observed in the hue of the samples. Lettuce is a complex system of enzymes, pigments and other compounds that affect the color of the product, such as peroxidase, poly phenoloxidase, ascorbic acid, carotenoids, and chlorophyll. Martín-Diana et al. (2007) reported that after treating iceberg lettuce with chlorine (120 ppm, 1 min) there was a decrease in the luminosity of the sample due to enzymatic browning. Similar results were observed in the present research for the chlorine treated samples. Samples presenting some changes in a^* value, moving to the reddish region might be attributed to some browning because of the degradation of green pigments such as chlorophylls (Martín-Diana et al., 2007). Lettuce treated with citric acid (1 and 2%) showed a^* values of −13.67 and −13.89, respectively, compared with the control −14.67, in which even though there was loss of greenness, the change was not statistically significant (Park et al., 2011). In Table 1, the sample treated with ultraviolet (0.65 mW/cm^2) showed an increase in redness, which could be due to a degradation of chlorophyll.

Lettuce treated with ozone showed a decrease of the overall visual quality after the concentration was higher than 2.5 ppm, showing a decrease in color; however, when samples were treated with 4 ppm and stored for 5 days some parts of the leafy structure lost the green color and showed a translucent appearance. This fact is explained in terms of the highly oxidant power of ozone that acts on the tissue of the lettuce and promotes the enzymatic activity of phenylalanine ammonia lyase (Ölmez & Akbas, 2009). Similar findings were found when iceberg lettuce was treated with ozone from 3 to 10 ppm (Koseki & Isobe, 2006).

The tomato sample presented minor changes in color parameters; luminosity was significantly different from the control in those samples that were treated with citric acid, regardless of the concentration. In general terms, citric acid promoted a light darkening of the tomatoes (from 46.66 to 40.24); redness was kept constant in almost all samples. Yellowness changed significantly ($p < 0.05$) between samples treated with citric acid, ultraviolet and ozone. The hue, which often is a quality indicator, was very close to zero (reddish region), but changed significantly ($p < 0.05$) between almost all the samples. In an experiment with red tomatoes treated with ultraviolet with a dose of 20 kJ/m^2 to extend their shelf-life, browning of the fruit was observed. However, when treated at 3 kJ/m^2 the tomato color index increased very quickly in the first week of storage, intensifying the red color of the product (Obande et al., 2011). The TCI observed in the present work changed significantly ($p < 0.05$) in the samples treated with citric acid (0.5%) and the lowest fluence of ultraviolet (0.65 mW/cm^2) compared with the control.

Finally, carrot also showed more changes in color after processing. Luminosity was significantly different ($p < 0.05$) in all samples compared with the control and the sample treated with ultraviolet; again, the highest fluence, was significantly different from almost all of the treated carrots, showing a higher L^* value. The redness–greenness of the samples was also affected. Samples moved down from a^* 32.86 (control) to values around 28, indicating a loss in redness. Hue and chroma again showed some significant differences ($p < 0.05$) between treatments. Chroma, which shows the degree of saturation, purity or intensity of color, changed for the samples treated with chlorine, citric acid and ultraviolet; in all cases, a reduction of the C^* value was observed.

In another study with ozone, carrots showed an increase in luminosity after processing and decrease in the chroma (Liew & Prange, 1994). Similar results were observed in the present work when carrots were treated with ozone. Carrots washed with distilled water and with citric acid (0.5%) did not show important changes in color; carrots treated with chlorine (200 ppm) during 2 min showed an increase in the whiteness index but differences were not significant ($p < 0.05$). The white discoloration of carrots is a product of an enzymatic reaction related with dehydration of the surface or formation of lignin (Amanatidou, Slump, Gorris, & Smid, 2000). In this research the change in the whiteness index was significantly different from the control for all of the evaluated treatments, showing in a minor or major degree of some kind of dehydration or enzymatic reaction. Samples treated with ultraviolet showed by far the highest change in WI and it was noticeable by sight after processing.

Table 1
Color parameters and color functions for lettuce, tomato and carrots after disinfection with selected treatments of chlorine, citric acid, ultraviolet light and ozone.

Treatment	L^*	a^*	b^*	ΔE	hue (h^*)	Chroma (C^*)	
	Lettuce						WI
Control	51.50 (±3.72)^{a}	−7.36 (±1.05)	16.31 (±3.08)		1.14 (±0.04)	2.97 (±0.37)	48.18 (±3.16)
Chlorine 50 ppm	48.11 (±5.56)^{b}	−8.22 (±1.08)^{a}	18.92 (±4.75)	9.27 (±3.10)	1.15 (±0.06)	3.22 (±0.60)	46.35 (±4.15)^{a}
Chlorine 100 ppm	48.68 (±3.36)^{c}	−7.79 (±1.21)	16.77 (±4.26)	6.92 (±3.88)	1.13 (±0.04)	2.96 (±0.51)	44.03 (±1.98)^{b}
Chlorine 200 ppm	45.64 (±3.11)^{d}	−7.18 (±1.26)	15.55 (±4.77)	7.73 (±4.62)	1.13 (±0.06)	2.84 (±0.59)^{a}	47.43 (±2.37)
Citric acid 0.5%	52.17 (±7.70)	−8.11 (±2.21)^{b}	20.16 (±9.44)	9.35 (±5.36)	1.16 (±0.10)	3.32 (±1.10)	43.85 (±3.87)^{c}
Citric acid 1%	47.07 (±2.30)^{e}	−7.77 (±1.37)	15.90 (±4.14)	6.02 (±2.69)	1.11 (±0.05)^{a}	2.81 (±0.50)^{b}	45.25 (±2.61)^{d}
Citric acid 1.5%	55.00 (±6.21)^{d}	−9.11 (±1.27)^{c}	23.87 (±7.97)^{a}	12.61 (±8.92)	1.19 (±0.08)	3.75 (±0.91)	42.82 (±3.29)^{e}
UV (0.65 mW/cm^2)	50.57 (±4.93)^{f}	−5.96 (±0.54)^{abcd}	14.74 (±2.99)^{ab}	6.51 (±3.34)	1.18 (±0.06)	2.93 (±0.48)	48.74 (±1.80)^{e}
UV (1.6 mW/cm^2)	51.94 (±2.71)	−6.86 (±0.73)^{c}	16.16 (±2.33)	4.86 (±3.38)^{a}	1.17 (±0.02)	3.04 (±0.27)	47.92 (±3.99)
Ozone 5 ppm	60.27 (±7.27)^{abcdef}	−8.52 (±0.63)^{d}	23.87 (±3.13)^{b}	13.45 (±5.97)^{a}	1.22 (±0.04)^{a}	3.90 (±0.39)^{ab}	52.51 (±4.97)^{abcde}
	Tomato						TCI
Control	46.66 (±1.31)^{a}	13.35 (±2.16)	9.91 (±1.41)		0.64 (±0.03)^{a}	4.81 (±0.34)	234.80 (±5.67)^{a}
Chlorine 50 ppm	42.53 (±4.25)	15.28 (±2.86)	10.22 (±2.03)	5.74 (±4.04)	0.59 (±0.05)^{b}	5.03 (±0.46)	242.27 (±8.87)
Chlorine 100 ppm	43.75 (±2.25)	14.63 (±2.98)	10.95 (±3.34)	5.08 (±3.10)	0.63 (±0.05)^{c}	5.03 (±0.61)	243.63 (±16.43)
Chlorine 200 ppm	43.41 (±2.75)	14.02 (±3.49)	10.01 (±2.76)	6.41 (±2.25)	0.62 (±0.06)	4.87 (±0.61)	241.60 (±19.43)
Citric acid 0.5%	42.09 (±2.86)^{a}	13.99 (±1.53)	11.15 (±2.53)	5.60 (±3.47)	0.67 (±0.05)^{d}	5.00 (±0.39)	255.26 (±8.96)^{a}
Citric acid 1%	40.24 (±2.38)^{a}	15.39 (±1.60)	12.81 (±2.95)^{a}	8.24 (±2.58)	0.69 (±0.08)^{be}	5.30 (±0.41)^{a}	243.76 (±10.69)
Citric acid 1.5%	41.09 (±3.70)^{a}	16.44 (±2.21)^{a}	13.37 (±1.74)^{b}	8.08 (±2.66)	0.68 (±0.08)^{f}	5.45 (±0.29)^{b}	246.98 (±13.70)
UV (0.65 mW/cm^2)	43.67 (±1.84)	13.67 (±1.79)	10.66 (±3.04)	5.27 (±3.40)	0.65 (±0.08)^{g}	4.91 (±0.49)	259.01 (±7.25)^{a}
UV (1.6 mW/cm^2)	44.29 (±1.21)	13.03 (±1.45)	7.70 (±1.36)^{ab}	4.46 (±2.73)	0.53 (±0.04)^{acdefg}	4.54 (±0.30)^{b}	240.29 (±11.51)
Ozone 5 ppm	44.10 (±1.45)	12.09 (±2.57)^{a}	7.83 (±1.70)^{ab}	4.94 (±2.88)	0.57 (±0.04)^{def}	4.44 (±0.47)^{ab}	252.74 (±7.75)
	Carrots						WI
Control	51.48 (±1.94)^{a}	32.86 (±1.95)^{a}	34.17 (±2.70)^{a}		0.80 (±0.02)	8.18 (±0.28)^{a}	32.14 (±3.27)^{a}
Chlorine 50 ppm	54.84 (±1.72)^{ab}	30.15 (±1.80)	29.30 (±2.85)	6.98 (±3.31)	0.77 (±0.02)^{a}	7.70 (±0.30)	40.09 (±2.43)^{a}
Chlorine 100 ppm	55.10 (±1.81)^{ac}	27.11 (±1.64)^{a}	28.88 (±2.43)	9.03 (±5.10)	0.82 (±0.01)^{ab}	7.48 (±0.27)^{a}	41.35 (±2.24)^{a}
Chlorine 200 ppm	56.88 (±1.40)^{ad}	28.59 (±1.70)^{a}	29.71 (±2.57)	8.87 (±4.85)	0.80 (±0.02)	7.63 (±0.28)	37.20 (±4.28)^{ab}
Citric acid 0.5%	54.80 (±1.86)^{ae}	27.36 (±2.11)^{a}	28.04 (±3.35)^{a}	9.40 (±3.93)	0.80 (±0.03)	7.43 (±0.37)^{a}	38.23 (±2.15)^{a}
Citric acid 1%	55.04 (±0.91)^{af}	26.23 (±2.41)^{a}	26.80 (±3.43)^{a}	10.94 (±4.08)	0.79 (±0.03)^{c}	7.27 (±0.39)^{a}	40.08 (±2.46)^{a}
Citric acid 1.5%	53.47 (±1.70)^{dg}	28.97 (±2.88)	30.42 (±4.80)	8.48 (±3.66)	0.81 (±0.03)^{ad}	7.69 (±0.49)	40.29 (±2.07)^{a}
UV (0.65 mW/cm^2)	57.22 (±2.21)^{ag}	28.38 (±2.99)^{a}	27.41 (±3.86)^{a}	10.61 (±4.88)	0.77 (±0.02)^{bde}	7.46 (±0.47)^{a}	42.59 (±3.23)^{abc}
UV (1.6 mW/cm^2)	59.01 (±1.47)^{abcefgh}	28.12 (±3.08)^{a}	28.50 (±3.76)	11.26 (±5.12)	0.79 (±0.02)^{f}	7.51 (±0.47)^{a}	41.65 (±2.77)^{a}
Ozone 5 ppm	54.65 (±1.54)^{ah}	28.90 (±2.11)^{a}	31.66 (±2.85)	6.88 (±3.04)	0.83 (±0.02)^{acef}	7.78 (±0.32)	37.55 (±2.80)^{ac}

Values are average ± standard deviation of at least two experiments. During each experiment at least five samples of each vegetable were taken. Each value was measured for at least four points in each vegetable.
Values with the same letter in the same column for each vegetable are significantly different ($p < 0.05$), Tukey's paired comparison.
The values represent the color parameters after the longest processing time (Chlorine 15 min; Citric acid 15 min; UV 60 min; Ozone 15 min).

3.6. Electron microscopy

In Fig. 6 the microstructure of carrots before and after treatment with ozone is shown. In Fig. 6a, the carrot tissue is shown free of any microorganism. However, Fig. 6b–d show how the cells of *E. coli* spread out into the tissue. Even though these images were taken after processing with ozone, bacterial cells seem to be attached to the tissue and some of them are protected under the folds of the tissue. According to microbiological counts, less than 1 log-reduction was achieved after 15 min of treatment with ozone,

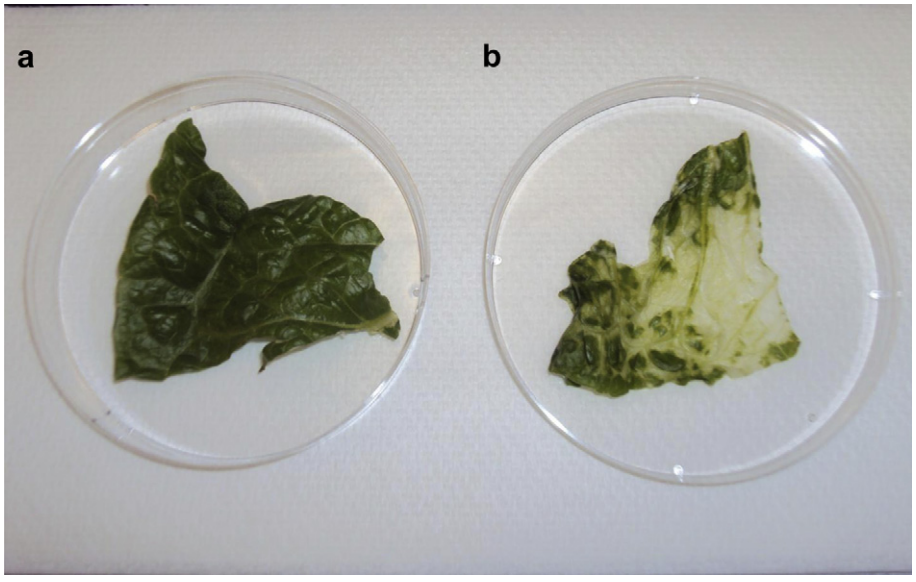

Fig. 5. Color in fresh romaine lettuce (a) and change in color after processing with ozone (5 ppm) during 15 min (b).

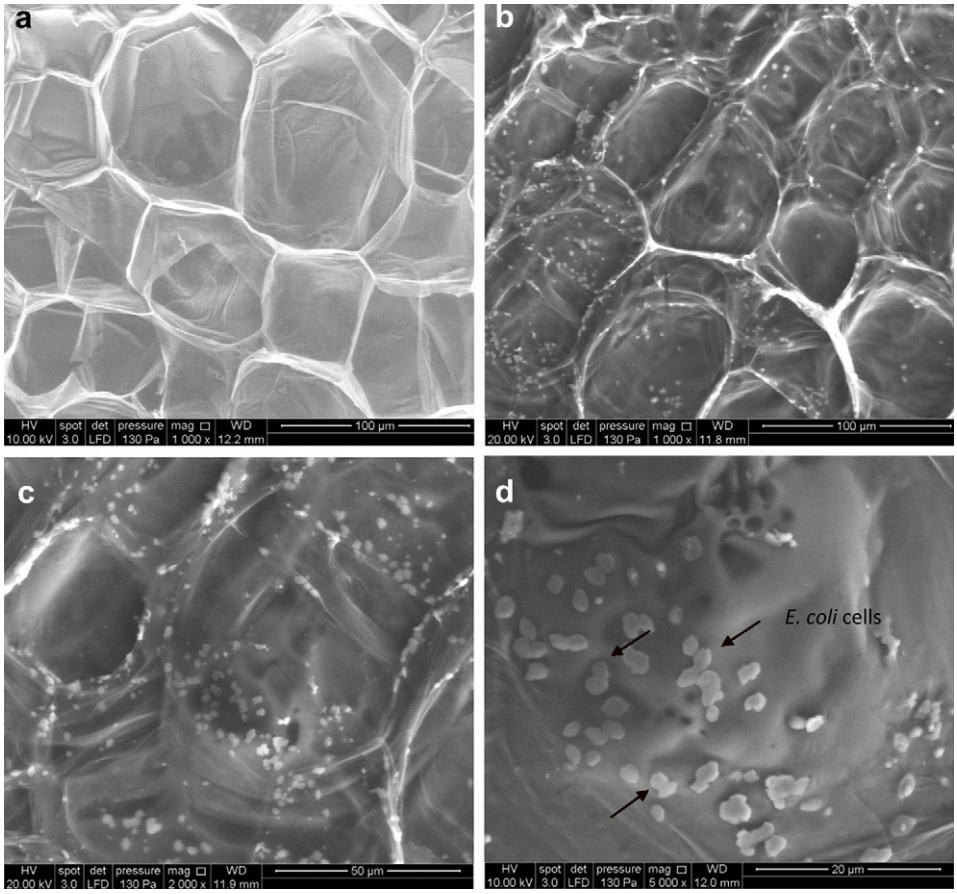

Fig. 6. Environmental scanning electron microscopy of carrots (a) before inoculation; (b–d) inoculated with *E. coli* and treated with ozone (5 ppm) by 15 min.

which means that most of the cells observed in these figures are alive. *E. coli* is characterized by having an intimate and strong attachment to the host cell membrane, even destroying the microvilli of the bacteria at the bonding site (Shaw et al., 2008). Topography of fruits and vegetables is a critical parameter for the adhesion of bacterial cells; some studies have shown that *E. coli* O157:H7 was better attached to coarse, porous or injured surfaces of green peppers, than to those without injuries (Wang, Feng, Liang, Luo, & Malyarchuk, 2009). Removal of *E. coli* from fresh produce with common water is not effective since cells are very well attached and only some disinfectant agents can reach the cells and inactivate them. It is difficult to removal bacterial cells because *E. coli* cells are added to leaves such as lettuce and spinach with EspA filaments, which are the same filaments used to attach to human and bovine cells, following a similar molecular mechanism as the one used to colonize the mammalian intestine (Shaw et al., 2008). In a very interesting study, four surface roughness's were evaluated in terms of adhesion of *E. coli* cells, showing that smooth surfaces (apple) are easy for bacterial removal, but when the roughness is higher with some deep valleys (oranges and avocados), bacteria is not totally exposed to the mechanical forces of washing. Finally, in products with high roughness with valleys and big cavities (cantaloupe) bacteria are well protected from mechanical forces and disinfection agents (Wang et al., 2009). Similar behavior was observed in the present work; bacteria inoculated in the smooth surface of tomatoes were easy to inactivate with some of the applied treatments, but when the surface roughness became more porous such as in carrots or lettuce, bacteria cells were well protected in the folds of the vegetable and the extent of disinfection was decreased.

4. Conclusions

The effectiveness of the three disinfection methods tested in this research were shown to be influenced by the dose of the agent, the exposure time and the surface of the food product. Smooth surface of vegetables such as tomatoes represents an easy product to allow direct contact of the sanitizer with the bacteria. When the surface becomes more complex in terms of porosity and roughness, the inactivation seems to be more complicated and reduced. Some changes in the color of produce can be controlled if the exposure time and/or concentration of the disinfection agent are kept as low as possible to inactivate the microorganism but still preserving the quality of the product.

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