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# Inactivation of *Escherichia coli* O157:H7, *Salmonella typhimurium*, and *Listeria monocytogenes* on cherry tomatoes and oranges by superheated steam

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ABSTRACT

This study was performed to compare the effectiveness of saturated steam and superheated steam for the inactivation of *Escherichia coli* O157:H7, *Salmonella typhimurium*, and *Listeria monocytogenes* on the surface of cherry tomatoes and oranges. It also determined the effect of the steam processes on the color, texture, Vitamin C content, and antioxidant capacity and changes in these parameters during chilled storage. Cherry tomatoes and oranges inoculated with the three foodborne pathogens were treated with saturated steam at 100 °C and superheated steam at 125, 150, 175, and 200 °C for various time intervals. After the cherry tomatoes and oranges were exposed to superheated steam at 200 °C for 3 or 20 s, all tested pathogens were reduced to below the detection limit (1 or 1.7 log, respectively) without significant changes in color, texture, vitamin C content, and antioxidant capacity ($P > .05$) at 4 °C for up to 9 days. Our results suggest that superheated steam treatment can be effective at decreasing pathogen populations when compared to saturated steam, without significant quality deterioration, and thus, this technique demonstrates great potential to improve the microbial safety of fresh produce.

## 1. Introduction

*Escherichia coli* O157:H7, *Salmonella typhimurium*, and *Listeria monocytogenes* have been implicated in foodborne outbreaks involving the consumption of fresh fruits and vegetables (Little & Gillespie, 2008; Sivapalasingam, Friedman, Cohen, & Tauxe, 2004). *E. coli* O157:H7 is an important pathogen capable of causing bloody diarrhea (hemorrhagic colitis) and renal failure (hemolytic uremic syndrome) in humans (Lim, Yoon, & Hovde, 2010). *S.* Typhimurium, a commonly isolated *Salmonella* serotype, has been implicated in foodborne illnesses characterized by diarrhea, abdominal pain, fever, chills, nausea, and vomiting (Coburn, Grassl, & Finlay, 2007). Listeriosis caused by L. *monocytogenes* can lead to abortion, neonatal death, septicemia, and meningitis (Schlech & Acheson, 2000).

In order to sanitize fresh produce, washing with chlorinated water has widely been used on a commercial scale to reduce the microbial load (Parish et al., 2003; Weissinger, Chantarapanont, & Beuchat, 2000). However, this treatment produces an antimicrobial effect of < 2 log CFU/g on fresh fruits and vegetables (Beuchat, 1999; Taormina & Beuchat, 1999) and is known to adversely react with organic matter, resulting in the formation of carcinogenic halogenated by-products (Hua & Reckhow, 2007). Furthermore, continuous exposure to chlorine-based sanitizers has the effect of increasing resistance of microorganisms (Davidson & Harrison, 2002). Furthermore, consumers prefer fresh produce that has not been treated with chemicals. Therefore, an alternative new method is needed to effectively reduce pathogens and simultaneously reduce or eliminate chemical use while still maintaining quality.

Recently, superheated steam (SHS) treatments have been evaluated for inactivating foodborne pathogens on chicken skin (Kondjoyan & Portanguen, 2008), almonds (Ban & Kang, 2016; Bari et al., 2010), pistachios (Ban & Kang, 2016), and biofilms on stainless steel and polyvinyl chloride (Ban, Yoon, & Kang, 2014). SHS is steam which is given additional heat to raise its temperature above the saturation temperature at a constant pressure (Cenkowski, Pronyk, Zmidzinska, & Muir, 2007) and has been known as a safe and non-polluting technology with low energy consumption (Chou & Chua, 2001). SHS has various advantages over other heating systems, including a high heat transfer rate due to condensation, an accelerated drying rate, and an oxygen-free environment (Bari et al., 2010). SHS treatment is able to transfer a large amount of latent heat to food when steam condenses on food surfaces because of the low initial temperature of a food material (Iyota,

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Nishimura, Yoshida, & Nomura, 2001; James, Goksoy, Corry, & James, 2000). Condensation of SHS occurs on food surfaces and then the condensed water evaporates back into the SHS because of its low moisture content, which leads to drying of the surface (Iyota, Nishimura, Yoshida, & Nomura, 2001).

Although several researchers observed that SHS heating has a strong killing effect against foodborne pathogens (Bulut, Purnell, James, Taylor, & Herbert, 2006), there have been few studies to demonstrate the inactivation effect of SHS on fresh produce. Because fresh produce is sensitive to heat, quality changes following thermal treatment with SS and SHS needs to be assessed. Therefore, the purpose of this study was to compare the bactericidal effectiveness of SS and SHS on cherry tomatoes and oranges, and investigate the quality changes following treatment with SS and SHS. To be specific, we evaluated the possibility of using SHS treatment on fresh produce and determined optimized treatment conditions to ensure both microbial safety and quality of cherry tomatoes and oranges.

## 2. Materials and methods

### 2.1. Bacterial strains and culture preparation

Three strains each of *E. coli* O157:H7 (ATCC 35150, ATCC 43889, ATCC 43890), *S.* Typhimurium (ATCC 19585, ATCC 43971, DT 104), and *L. monocytogenes* (ATCC 15315, ATCC 19114, ATCC 19115) were obtained from the bacterial culture collection at Seoul National University (Seoul, Korea) and used in this study. Stock cultures were stored at −80 °C in 0.7 mL of tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks, MD, USA) and 0.3 mL of 50% glycerol. Working cultures were streaked onto tryptic soy agar (TSA; Difco), incubated at 37 °C for 24 h, and stored at 4 °C. Each strain of *E. coli* O157:H7, *S.* Typhimurium, and L. *monocytogenes* was grown in 10 mL of TSB at 37 °C for 24 h. Cells of each strain were collected by centrifugation at 4000 *g* at 4 °C for 20 min and washed three times with buffered peptone water (BPW; Difco, Sparks, MD). The final pellets were resuspended in sterile BPW, corresponding to approximately $10^{10}$ CFU/mL. Suspended pellets of the three strains of each pathogen were combined to produce a mixed culture cocktail.

### 2.2. Sample preparation and inoculation procedure

Fresh unblemished and uncoated cherry tomatoes (*Lycopersicon esculentum* Mill.) and Valencia oranges (*Citrus sinensis*) were supplied by a local market (Seoul, Korea) on the day before the experiment and stored at 4 °C prior to use. The cherry tomatoes and oranges were 3 ± 0.1 cm and 8.5 ± 0.2 cm in diameter and 25 ± 1 g and 100 ± 5 g, respectively ($n$ = 100).

Cherry tomatoes and oranges were placed on sterile aluminum foils in a laminar flow hood and spot-inoculated with 0.1 mL of the culture cocktail by depositing droplets with a micropipette at 10 to 15 locations on the surface. Inoculated cherry tomatoes and oranges were then air-dried for 1 h in the hood with the fan running at 22 ± 2 °C.

### 2.3. Saturated steam and superheated steam treatment

The experimental apparatus consisted of a saturated steam generator with a maximum power of 5 kW at a 220 V input, a superheated steam generator with a maximum power of 6 kW at a 220 V input and an insulated sample treatment stainless steel chamber (external diameter, 23 cm; external height, 32 cm; internal diameter, 17 cm; internal height, 22.5 cm), and a flexible stainless steel connection hose (Fig.1). SS at 100 °C, produced by the SS generator, was introduced into the SHS generator through a flexible tube. SS was converted into SHS by heating with an electrical resistance heater in the SHS generator. The maximum temperature generated from the SHS generator used in this study was 200 °C, which was made by giving additional heat to SS at constant

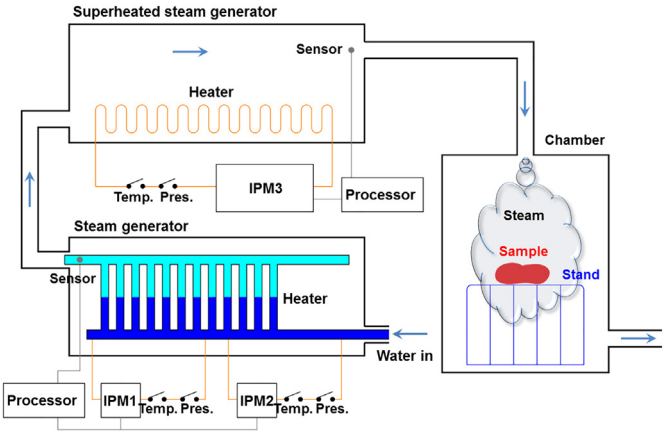


**Fig. 1.** Schematic diagram of the superheated steam treatment system used in this study.

pressure. During these experiments, the SS and SHS temperature was controlled automatically by means of a temperature sensor and an intelligent power module (IPM) in each of the steam generators. After SS and SHS temperature of the inlet into the chamber had stabilized (following 5 min warm-up time), two inoculated cherry tomatoes or an inoculated orange were placed on a stainless steel treatment grid (9 by 9 by 10 cm). A manual valve that was placed on top of the treatment chamber took 0.1–0.2 s to open and close and was used to control the steam flow. The lid of the chamber allowed samples to be inserted and removed within 2 s. Steam passed through the flexible hose into the chamber by opening the steam valve and the velocity of steam was 5.0 m/s.

Cherry tomatoes and oranges were steam treated for 1, 2, 3, 4, and 5 s and 1, 5, 10, 20, and 30 s, respectively. SS treatment was performed at 100 °C while SHS treatments were performed at 125, 150, 175, and 200 °C. For the microbial test and quality test, the treated cherry tomato or orange samples were immediately removed from the chamber after each treatment and placed in a stomacher bag (Labplas Inc., Sainte-Julie, Quebec, Canada) containing 225 or 50 mL of 0.2% peptone water, respectively, and then the bags were put in ice water to reduce the remaining heat.

A fiber optic temperature sensor (FOT-L; FISO Technologies Inc., Quebec, Canada) connected to a temperature signal conditioner was used to measure real-time temperatures in each sample during the SS and SHS treatments. The sensors were placed directly on the surface of the non-inoculated cherry tomatoes and oranges, as well as 1 cm away from the chamber wall in the surrounding environment.

### 2.4. Bacterial enumeration

The treated (SS and SHS) or non-treated samples were stored at refrigerated temperature (4 ± 2 °C) for 0, 1, 3, 5, 7, and 9 days, and were analyzed for the survival of three pathogens. Non-treated samples were used as a control. The samples were combined with 225 or 50 mL of TSB with 2% NaCl for determination of surviving bacteria. Steam treated cherry tomato samples were homogenized at 230 rpm for 2 min with a mechanical stomacher (EASY MIX, AES Chemunex, Rennes, France) and orange samples were shaken and massaged by hands for 1 min. After homogenization for the cherry tomatoes and massage for the orange, 1 mL aliquots of samples were 10-fold serially diluted with 9 mL of sterile 0.2% peptone water, and 0.1 mL of appropriate dilutions were spread-plated onto Sorbitol MacConkey Agar (SMAC; Difco), Xylose Lysine Desoxycholate Agar (XLD; Difco), and Oxford Agar Base (OAB; Difco) with antimicrobic supplement (Difco) to enumerate surviving populations of *E. coli* O157:H7, *S.* Typhimurium, and L. *monocytogenes*, respectively. All plates were incubated at 37 °C for 24 h and

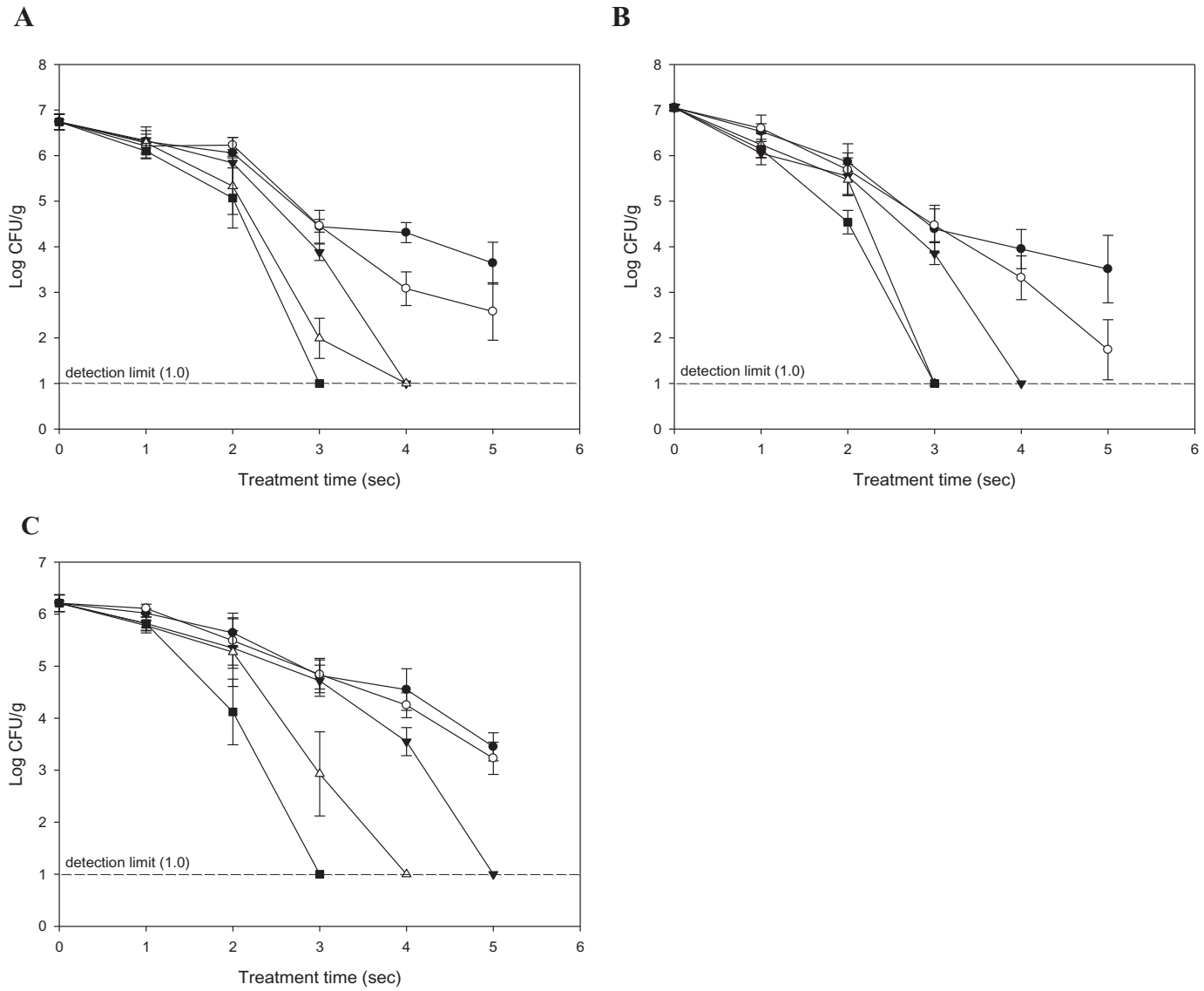


**Fig. 2.** Survival curves for *E. coli* O157:H7 (A), *S.* Typhimurium (B), and L. *monocytogenes* (C) on cherry tomatoes treated with SS at 100 °C (●), SHS at 125 °C (○), SHS at 150 °C (▼), SHS at 175 °C (△), SHS at 200 °C (■).

colonies were enumerated. To confirm pathogen identity, presumptive colonies were randomly picked from selective media and subjected to biochemical and serological tests. These tests consisted of the *E. coli* O157:H7 latex agglutination assay (Oxoid, Basingstoke, UK), the *Salmonella* latex agglutination assay (Oxoid, Basingstoke, UK), and the API *Listeria* test (BioMérieux, Hazelwood, MO).

### 2.5. Color and texture measurement

To determine the effect of SS and SHS treatment on the color of cherry tomatoes and oranges, color assessments were carried out using a Minolta colorimeter (Model CR-400; Minolta Camera Co. Ltd., Osaka, Japan). After treated samples were cooled in a stomacher bag surrounded by ice water, measurements on all samples were conducted at random locations on cherry tomatoes and oranges and averaged. L*, a*, and b* values indicate lightness, redness, and yellowness of the sample, respectively.

Changes in texture of SS and SHS treated cherry tomatoes and oranges were assessed with a texture analyzer (TA-CT3, Brookfield Engineering Laboratories, Inc., Middleboro, MA, USA) with a cylinder probe. To evaluate the cherry tomatoes and oranges, a cooled sample was placed with its side faced up onto the press holder, and a 1.5 or 4 mm diameter cylinder blade was set at speed of 2 mm/s to compress the fruit 10 and 25 mm from the contact point, respectively. Maximum force was recorded using TexturePro CT software (Brookfield Engineering Laboratories, Inc.). Three measurements were performed for each treatment with independently-prepared samples. The color and texture of the control and all treated samples were measured after 1, 3, 5, 7, and 9 days of refrigerated storage (4 ± 2 °C).

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### 2.6. Vitamin C measurement

Vitamin C content in cherry tomatoes and oranges was determined following the method validated by Odriozola-Serrano, Hernández-Jover, and Martín-Belloso (2007) with minor modifications. Also, ascorbic acid contents in orange peels were investigated to observe the heat effect of steam on the fruits surface. Individually, treated samples were homogenized at 7 °C using a homogenizer (WiseMix HG-15D, Daihan Scientific Co., Ltd., Korea) at 3000 rpm for 3 min for the cherry tomatoes and an electric blender (Tefal BL16, France) at a maximum speed for 3 min for the orange pulp and orange peels. And, a 15 mL

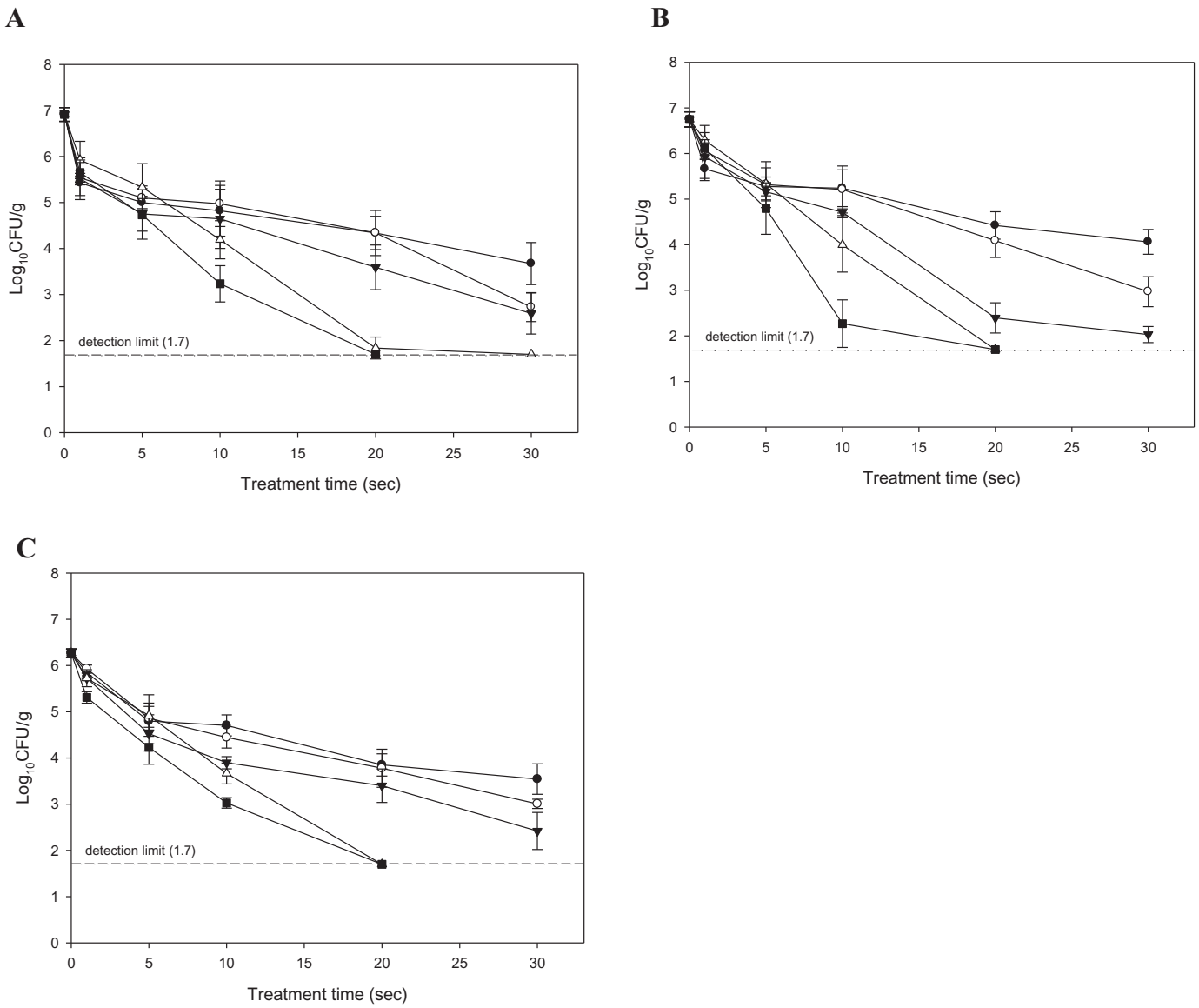


**Fig. 3.** Survival curves for *E. coli* O157:H7 (A), *S.* Typhimurium (B), and *L. monocytogenes* (C) on oranges treated with SS at 100 °C (●), SHS at 125 °C (○), SHS at 150 °C (▼), SHS at 175 °C (△), SHS at 200 °C (■).

sample was mixed with 15 mL of solution containing 45 g/L metaphosphoric acid and 7.2 g/L dithiothreitol. The resulting mixture was centrifuged at 15,300 *g* at 4 °C for 15 min and a 10 μL aliquot of the supernatant was injected into a high-performance liquid chromatography (HPLC; Ultimate 3000; Dionex, Sunnyvale, CA) equipped with an autosampler and an UV detector set at 254 nm. A reversed-phase C18 column (5-μm particle size, 4.6-mm diameter, 250-mm length; Dionex) was used to separate the ascorbic acid using 50 mM potassium phosphate buffer (pH 7.2) and acetonitrile (95:5 [vol/vol]) as a mobile phase. The mobile phase was filtered using a 0.45 μm pore size membrane filter (Micron Separations, Inc., Westboro, MA) and degassed using a vacuum before being applied to the column. A flow rate of 0.5 mL/min was used, and the retention time was 3.7 min. A standard calibration curve was obtained by using L-ascorbic acid (Sigma Chemical Co., St. Louis, MO) in concentrations ranging from 5 to 80 mg/100 mL.

### 2.7. Determination of antioxidant capacity

The antioxidant capacities of cherry tomatoes, orange pulp, and orange peels were assayed through evaluation of the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging effect. This determination was based on the method validated by De Ancos, Sgroppo, Plaza, and Cano (2002). Individually, treated samples were homogenized and were centrifuged at 6000 *g* at 4 °C for 15 min, and a reaction mixture of aliquots (0.010 mL) of the sample supernatant in 3.9 mL of methanolic DPPH· (0.025 g/L) and 0.090 mL of distilled water were shaken vigorously and kept in darkness for 30 min. The absorption of the samples was measured spectrophotometrically using a microplate reader (Spectramax M2e; Molecular Devices, Sunnyvale, CA) at 517 nm. Results were expressed as the percentage of inhibition of the radical DPPH·, that is, the decrease in absorbance with respect to the control value (DPPH· initial absorption value).

### 2.8. Statistical analysis

All experiments were repeated three times with duplicate samples. Triplicate data were analyzed by analysis of variance (ANOVA) using the Statistical Analysis System (SAS Institute, Cary, NC, USA) and the separation of means was tested by Duncan's multiple-range test at a

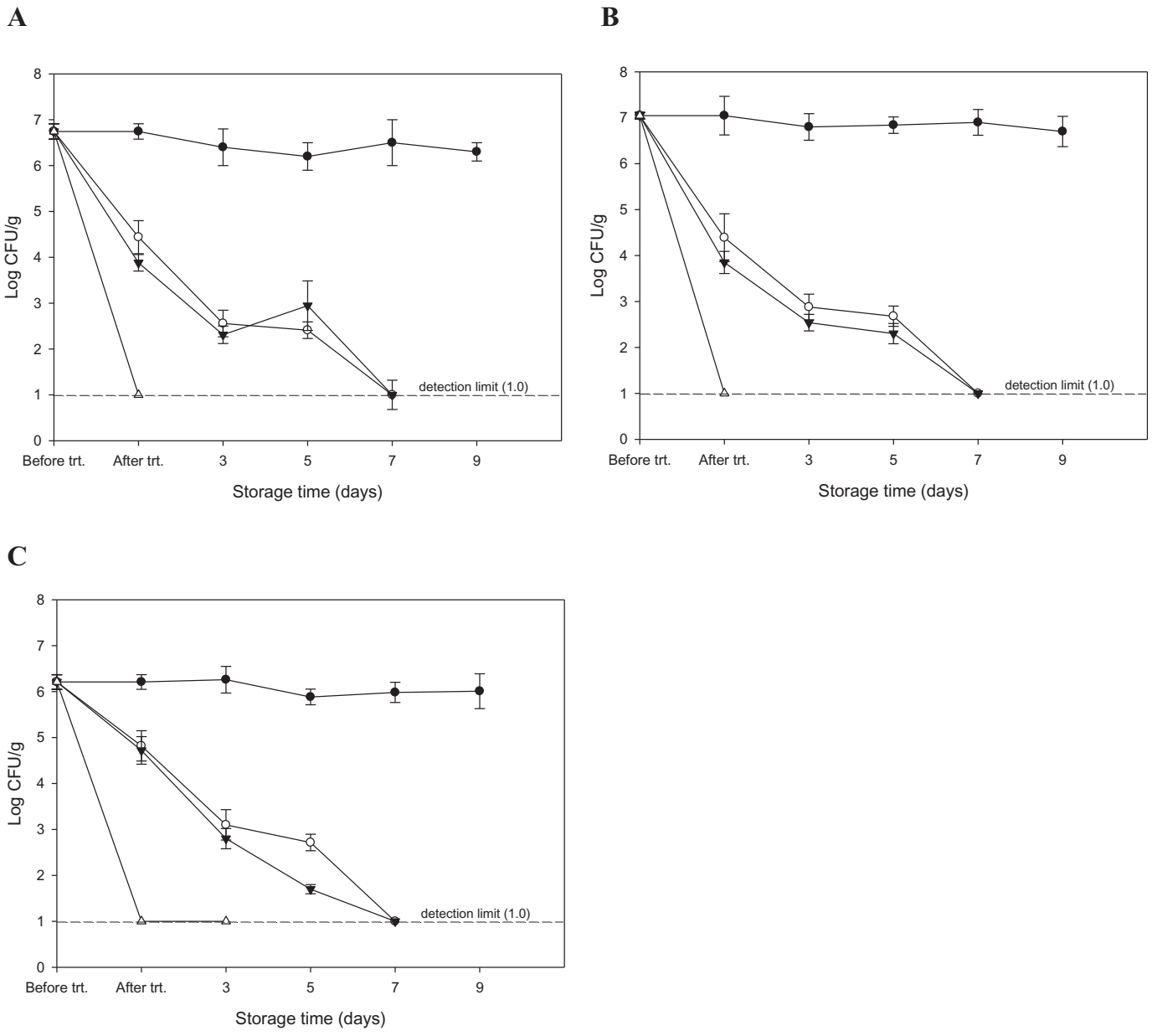


**Fig. 4.** Changes in the populations of *E. coli* O157:H7 (A), *S.* Typhimurium (B), and *L. monocytogenes* (C) on cherry tomatoes untreated (●), treated with SS at 100 °C for 3 s (○), SHS at 150 °C for 3 s (▼), and SHS at 200 °C for 3 s (△) at 4 °C for up to 9 days.

probability level of $P < .05$.

## 3. Results

### 3.1. Inactivation of pathogenic bacteria on cherry tomatoes and oranges

During steam treatment at 100, 150, and 200 °C, the average surface temperatures of the fruits were 72, 96, and 138 °C and the environment temperatures surrounding the fruits were 97, 141, and 186 °C, respectively. After subsequent cooling in the ice water, the average surface temperature was decreased to 29 °C. Populations (log CFU/g) of *E. coli* O157:H7, *S.* Typhimurium, and L. *monocytogenes* on cherry tomatoes and oranges following SS and SHS treatment are depicted in Figs. 2 and 3. Initial inoculum levels of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* on cherry tomatoes were 6.74, 7.05, and 6.21 log CFU/g and those on oranges were 6.91, 6.75, and 6.27 log CFU/g, respectively. As the temperature and duration of SHS treatment increased, surviving populations of the three pathogens decreased more dramatically. After SHS treatment at 200 °C for 3 s, levels of the three pathogens on cherry tomatoes were below the detection limit (1 log CFU/g), whereas SS treatment at 100 °C reduced to achieved only 4.44, 4.39, and 4.82 log of *E. coli* O157:H7, *S.* Typhimurium, and L. *monocytogenes*, respectively. Populations of the three pathogens on oranges were greatly reduced to undetectable levels (1.7 log CFU/g) when treated with SHS treatment at 200 °C for 20 s, while the populations of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* reduced of 4.34, 4.42, and 3.85 after SS treatment at 100 °C for the same time interval. It was observed that SHS treatment caused additional 3.39–3.82 and 2.15–2.72 log reductions of the three pathogens on cherry tomatoes and oranges, respectively, compared to SS treatments. In addition, populations of the three pathogens on cherry tomatoes were reduced to below the detection limit when subjected to heating for 4 s at 150 °C and at 175 °C, 4 s at 150 °C and 3 s at 175 °C, and 5 s at 150 °C and 4 s at 175 °C for *E. coli* O157:H7, *S.* Typhimurium, and L. *monocytogenes,* respectively. In the case of

**Table 1**
Color analysis of steam treated cherry tomatoes (A) and oranges (B) where L* is lightness, a* is redness, and b* is yellowness.

(A) Cherry tomatoes

| Color Parameter | Temperature (°C) | Steam duration (s) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 4 | 5 |
| L* | 25 | 37.97 ± 1.55 A | – | – | – | – | – |
| | 100 | – | 37.52 ± 0.63 A | 37.92 ± 0.91 A | 38.03 ± 1.73 A | 38.19 ± 0.95 A | 37.96 ± 0.80 A |
| | 125 | – | 37.07 ± 1.30 A | 37.61 ± 0.43 A | 38.54 ± 1.08 A | 38.43 ± 1.19 A | 38.32 ± 1.01 A |
| | 150 | – | 37.39 ± 0.80 A | 38.19 ± 0.57 A | 37.91 ± 0.66 A | 38.74 ± 1.46 A | 38.25 ± 1.47 A |
| | 175 | – | 38.00 ± 0.79 A | 38.37 ± 1.68 A | 37.70 ± 0.94 A | 37.52 ± 1.12 A | 38.19 ± 0.41 A |
| | 200 | – | 38.46 ± 1.11 A | 37.47 ± 0.90 A | 37.65 ± 0.66 A | 38.28 ± 0.42 A | 38.10 ± 0.99 A |
| a* | 25 | 15.82 ± 0.87 A | – | – | – | – | – |
| | 100 | – | 15.53 ± 0.45 A | 15.30 ± 0.49 A | 15.32 ± 0.67 A | 15.79 ± 0.59 A | 14.70 ± 0.73 A |
| | 125 | – | 15.71 ± 0.78 A | 15.58 ± 0.13 A | 16.31 ± 0.48 A | 16.23 ± 0.92 A | 16.01 ± 0.45 A |
| | 150 | – | 15.83 ± 0.82 A | 15.62 ± 0.94 A | 16.38 ± 0.81 A | 16.16 ± 0.32 A | 15.86 ± 0.24 A |
| | 175 | – | 15.81 ± 0.38 A | 15.64 ± 0.51 A | 16.33 ± 0.55 A | 16.24 ± 0.94 A | 16.34 ± 0.34 A |
| | 200 | – | 15.42 ± 0.99 A | 15.53 ± 0.24 A | 16.51 ± 0.39 A | 16.26 ± 0.89 A | 16.13 ± 0.55 A |
| b* | 25 | 18.00 ± 0.37 A | – | – | – | – | – |
| | 100 | – | 18.75 ± 0.42 A | 18.23 ± 0.41 A | 18.04 ± 0.12 A | 18.39 ± 0.38 A | 18.42 ± 0.43 A |
| | 125 | – | 17.49 ± 0.72 A | 18.18 ± 0.32 A | 18.52 ± 0.72 A | 18.89 ± 0.66 A | 18.73 ± 0.67 A |
| | 150 | – | 18.35 ± 0.44 A | 18.54 ± 0.64 A | 18.11 ± 0.13 A | 18.93 ± 0.82 A | 18.89 ± 0.62 A |
| | 175 | – | 18.86 ± 0.77 A | 18.25 ± 0.21 A | 18.31 ± 0.22 A | 18.04 ± 0.31 A | 18.25 ± 0.29 A |
| | 200 | – | 18.07 ± 0.26 A | 18.18 ± 0.28 A | 18.88 ± 0.63 A | 18.54 ± 0.22 A | 18.41 ± 0.55 A |

(B) Oranges

| Color Parameter | Temperature (°C) | Steam duration (s) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 5 | 10 | 20 | 30 |
| L* | 25 | 71.75 ± 1.58 A | – | – | – | – | – |
| | 100 | – | 70.58 ± 1.89 A | 71.50 ± 0.63 A | 70.51 ± 1.31 A | 72.02 ± 1.32 A | 71.05 ± 1.59 A |
| | 125 | – | 71.45 ± 1.13 A | 72.49 ± 0.96 A | 71.26 ± 1.35 A | 72.29 ± 1.24 A | 72.11 ± 1.49 A |
| | 150 | – | 71.79 ± 0.90 A | 71.71 ± 1.74 A | 71.63 ± 0.55 A | 70.59 ± 1.77 A | 72.19 ± 1.47 A |
| | 175 | – | 70.28 ± 1.70 A | 71.06 ± 0.64 A | 71.75 ± 1.18 A | 71.43 ± 0.55 A | 70.55 ± 1.07 A |
| | 200 | – | 72.03 ± 1.40 A | 73.08 ± 1.87 A | 70.64 ± 1.42 A | 71.52 ± 0.68 A | 71.99 ± 0.68 A |
| a* | 25 | 10.70 ± 1.03 A | – | – | – | – | – |
| | 100 | – | 9.77 ± 1.32 A | 9.34 ± 1.85 A | 9.31 ± 1.86 A | 10.42 ± 1.41 A | 9.09 ± 2.11 A |
| | 125 | – | 9.71 ± 1.23 A | 9.21 ± 1.31 A | 9.65 ± 1.43 A | 9.82 ± 2.24 A | 10.71 ± 0.61 A |
| | 150 | – | 9.06 ± 1.76 A | 10.75 ± 1.00 A | 10.23 ± 1.11 A | 9.68 ± 1.83 A | 8.40 ± 2.95 A |
| | 175 | – | 10.35 ± 1.26 A | 10.69 ± 0.89 A | 9.80 ± 1.10 A | 9.63 ± 1.20 A | 9.23 ± 1.55 A |
| | 200 | – | 9.13 ± 1.75 A | 9.33 ± 1.55 A | 11.35 ± 1.86 A | 9.81 ± 1.84 A | 9.41 ± 1.85 A |
| b* | 25 | 65.72 ± 1.98 A | – | – | – | – | – |
| | 100 | – | 64.87 ± 1.39 A | 65.23 ± 1.20 A | 64.59 ± 1.85 A | 64.30 ± 1.48 A | 66.37 ± 2.04 A |
| | 125 | – | 66.47 ± 1.41 A | 65.55 ± 1.17 A | 64.16 ± 2.47 A | 65.48 ± 0.57 A | 66.69 ± 2.01 A |
| | 150 | – | 66.75 ± 1.33 A | 65.26 ± 1.63 A | 64.10 ± 1.81 A | 64.78 ± 1.63 A | 64.28 ± 2.67 A |
| | 175 | – | 65.33 ± 0.99 A | 66.98 ± 2.34 A | 66.00 ± 0.67 A | 64.57 ± 1.46 A | 64.26 ± 1.87 A |
| | 200 | – | 66.00 ± 1.26 A | 64.36 ± 2.22 A | 65.57 ± 1.10 A | 65.21 ± 0.63 A | 64.81 ± 2.33 A |

Values are means ± standard deviations from three replications.
Means followed by the same letter in a data series (column) are not significantly different ($P > .05$).

oranges, numbers of the three pathogens were reduced to below the detection limit when treated for 30 s at 175 °C, 20 s at 175 °C, and 20 s at 175 °C for *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes,* respectively.

The effectiveness of SS and SHS treatments for reduction of three pathogens on cherry tomatoes and oranges at 4 °C for up to 9 days of storage was tested, and results are shown in Fig. 4. There were no significant ($P > .05$) reductions of the three pathogens populations during 9 day storage on untreated cherry tomatoes and oranges (controls). Bacterial cell counts of cherry tomatoes were reduced to below the detection limit (1 log CFU/g) when subjected to heating at 100 and 150 °C for 3 s after 7 day storage and at 200 °C for 3 s immediately after treatment. The time required to reduce three foodborne pathogens to undetectable levels (1.7 log CFU/g) on oranges was 5, 5 and 0 days (immediately after treatment) at 100, 150, and 200 °C for 20 s, respectively (data not shown).

### 3.2. Effect of SS and SHS treatment on color and texture of cherry tomatoes and oranges

The color values (L*, a*, and b*) of cherry tomatoes and oranges after SS and SHS treatment are summarized in Table 1. The color values for SS and SHS treated cherry tomatoes and oranges were not significantly ($P > .05$) different from those of untreated samples. Differences in color values were insignificant ($P > .05$) in all cherry tomatoes at different treatments over the storage period (Table 5). Although measured three color values of samples were irregular due to natural color variations in fresh produce, statistically significant differences were not observed during the entire treatment interval.

Also, SS and SHS treatment duration for 5 s on cherry tomatoes and 30 s on oranges did not significantly ($P > .05$) change the maximum load values of the texture measurements (Table 2). However, time-dependent significant ($P < .05$) differences in maximum load values were observed in cherry tomatoes during the storage and no significant ($P > .05$) decreases were observed between untreated and steam treated samples (Table 6).

**Table 2**
Maximum load values for texture of cherry tomatoes (A) and oranges (B) following treatment with SS and SHS.

(A) Cherry tomatoes

| | Steam duration (s) | | | | | |
|---|---|---|---|---|---|---|
| Temperature (°C) | 0 | 1 | 2 | 3 | 4 | 5 |
| 25 | 16.52 ± 1.43 A | – | – | – | – | – |
| 100 | – | 16.05 ± 1.11 A | 16.76 ± 1.18 A | 16.43 ± 0.92 A | 16.59 ± 0.68 A | 16.22 ± 0.92 A |
| 125 | – | 16.26 ± 1.42 A | 16.25 ± 0.42 A | 16.43 ± 1.42 A | 16.69 ± 0.49 A | 16.24 ± 1.32 A |
| 150 | – | 16.86 ± 1.21 A | 16.18 ± 0.82 A | 16.78 ± 1.04 A | 16.58 ± 1.12 A | 16.29 ± 1.04 A |
| 175 | – | 16.95 ± 1.08 A | 16.56 ± 0.22 A | 16.55 ± 0.53 A | 16.14 ± 0.97 A | 16.78 ± 1.23 A |
| 200 | – | 16.52 ± 0.59 A | 16.85 ± 0.84 A | 16.43 ± 0.77 A | 16.27 ± 0.66 A | 16.18 ± 0.95 A |

(B) Oranges

| | 0 | 1 | 5 | 10 | 20 | 30 |
|---|---|---|---|---|---|---|
| 25 | 37.60 ± 3.44 A | – | – | – | – | – |
| 100 | – | 35.90 ± 3.90 A | 38.82 ± 4.40 A | 38.23 ± 3.92 A | 38.37 ± 5.35 A | 36.92 ± 3.95 A |
| 125 | – | 40.39 ± 4.11 A | 38.00 ± 3.11 A | 37.77 ± 3.21 A | 38.78 ± 4.20 A | 36.88 ± 5.45 A |
| 150 | – | 39.22 ± 2.51 A | 35.97 ± 5.85 A | 35.55 ± 4.73 A | 40.40 ± 5.93 A | 36.19 ± 4.11 A |
| 175 | – | 35.00 ± 2.17 A | 35.68 ± 3.73 A | 37.10 ± 4.89 A | 36.89 ± 3.71 A | 39.20 ± 4.19 A |
| 200 | – | 40.15 ± 4.30 A | 35.05 ± 3.90 A | 35.45 ± 3.30 A | 37.03 ± 4.37 A | 37.49 ± 3.55 A |

Values are means ± standard deviations from three replications.
Means followed by the same letter in a data series (column) are not significantly different ($P > .05$).

**Table 3**
Vitamin C contents of cherry tomatoes (A), orange pulp (B), and orange peel (C) following treatment with SS and SHS.

(A) Cherry tomatoes

| Temperature (°C) | Steam duration (s) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 |
| 25 | 21.02 ± 1.84 A | – | – | – | – | – |
| 100 | – | 20.21 ± 1.12 A | 20.32 ± 1.02 A | 19.02 ± 1.77 A | 19.03 ± 1.79 A | 19.02 ± 1.91 A |
| 125 | – | 22.32 ± 1.58 A | 19.47 ± 1.97 A | 21.67 ± 0.73 A | 20.29 ± 1.32 A | 19.63 ± 1.58 A |
| 150 | – | 19.89 ± 1.55 A | 19.36 ± 1.79 A | 20.29 ± 0.83 A | 20.74 ± 0.48 A | 18.89 ± 2.42 A |
| 175 | – | 21.39 ± 0.87 A | 20.83 ± 0.54 A | 23.17 ± 2.01 A | 19.34 ± 1.69 A | 20.03 ± 1.79 A |
| 200 | – | 20.32 ± 1.38 A | 18.32 ± 2.10 A | 19.66 ± 1.64 A | 20.21 ± 1.42 A | 20.40 ± 1.82 A |

(B) Orange pulp

| Temperature (°C) | Steam duration (s) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 1 | 5 | 10 | 20 | 30 |
| 25 | 48.90 ± 4.23 A | – | – | – | – | – |
| 100 | – | 53.06 ± 5.22 A | 48.44 ± 5.62 A | 43.79 ± 5.74 A | 58.86 ± 3.21 B | 53.48 ± 4.23 AB |
| 125 | – | 49.52 ± 3.83 A | 47.34 ± 4.23 A | 47.83 ± 3.18 A | 45.12 ± 4.23 A | 47.67 ± 2.97 A |
| 150 | – | 48.32 ± 1.83 A | 57.41 ± 2.29 B | 45.86 ± 4.22 A | 50.24 ± 3.22 A | 59.00 ± 3.11 B |
| 175 | – | 47.59 ± 2.11 A | 56.73 ± 1.98 B | 46.72 ± 2.27 A | 48.03 ± 2.98 A | 46.81 ± 4.74 A |
| 200 | – | 45.67 ± 4.49 A | 44.94 ± 4.23 A | 53.99 ± 4.68 AB | 45.99 ± 3.85 A | 46.48 ± 3.19 A |

(C) Orange peels

| | 0 | 1 | 5 | 10 | 20 | 30 |
|---|---|---|---|---|---|---|
| 25 | 28.29 ± 3.89 A | – | – | – | – | – |
| 100 | – | 25.97 ± 3.39 A | 24.02 ± 5.73 A | 24.60 ± 2.34 AB | 23.80 ± 6.18 A | 22.25 ± 1.98 B |
| 125 | – | 26.82 ± 3.70 A | 25.22 ± 3.66 A | 24.53 ± 3.44 A | 22.08 ± 3.28 AB | 26.69 ± 2.19 A |
| 150 | – | 26.35 ± 2.64 A | 25.95 ± 2.74 A | 23.60 ± 5.89 A | 26.34 ± 1.89 A | 21.49 ± 1.15 B |
| 175 | – | 27.52 ± 1.42 A | 24.99 ± 3.88 A | 24.79 ± 4.21 A | 24.53 ± 4.33 AB | 22.47 ± 4.42 A |
| 200 | – | 26.51 ± 2.41 A | 29.87 ± 1.28 A | 27.47 ± 2.19 A | 23.07 ± 3.28 AB | 22.15 ± 2.87 B |

The values are means ± standard deviations from three replications.
Means followed by the same letter in a data series (column) are not significantly different ($P > .05$).

Peeling of tomato skin occurred when both SS and SHS treatment times exceeded 5 s. The cherry tomatoes' color and texture were changed when the superheated steam treatment time exceeded 5 s because of the high temperature.

### *3.3. Effect of SS and SHS treatment on vitamin C and antioxidant capacities of cherry tomatoes, orange pulp, and orange peels*

The vitamin C content of untreated cherry tomatoes, orange pulp,

**Table 4**
Effects of superheated steam on antioxidant capacity of cherry tomatoes (A), orange pulp (B), and orange peel (C) following treatment with SS and SHS.

(A) Cherry tomatoes

| Temperature (°C) | Steam duration (s) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 |
| 25 | 86.64 ± 4.85 A | – | – | – | – | – |
| 100 | – | 86.79 ± 3.07 A | 88.31 ± 2.10 A | 86.73 ± 3.18 A | 85.94 ± 4.25 A | 87.71 ± 1.77 A |
| 125 | – | 86.63 ± 2.22 A | 87.07 ± 2.94 A | 85.68 ± 3.76 A | 86.51 ± 3.49 A | 87.06 ± 2.21 A |
| 150 | – | 88.62 ± 2.63 A | 86.58 ± 4.32 A | 86.52 ± 3.68 A | 86.15 ± 4.74 A | 85.75 ± 4.91 A |
| 175 | – | 86.91 ± 3.23 A | 85.47 ± 5.58 A | 85.98 ± 3.73 A | 85.53 ± 4.49 A | 86.14 ± 4.36 A |
| 200 | – | 85.99 ± 4.27 A | 86.02 ± 4.83 A | 86.09 ± 4.5 A | 87.17 ± 2.99 A | 85.63 ± 4.92 A |

(B) Orange pulp

| Temperature (°C) | Steam duration (s) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 1 | 5 | 10 | 20 | 30 |
| 25 | 89.42 ± 3.19 A | – | – | – | – | – |
| 100 | – | 88.02 ± 2.91 A | 89.95 ± 3.92 A | 89.54 ± 2.85 A | 88.85 ± 1.11 A | 88.81 ± 2.19 A |
| 125 | – | 89.09 ± 2.22 A | 90.12 ± 1.97 A | 88.53 ± 2.95 A | 88.18 ± 2.84 A | 89.95 ± 2.75 A |
| 150 | – | 89.44 ± 1.94 A | 89.40 ± 1.65 A | 90.49 ± 1.89 A | 89.04 ± 1.81 A | 90.27 ± 1.74 A |
| 175 | – | 88.96 ± 3.39 A | 89.28 ± 0.89 A | 89.29 ± 2.65 A | 89.47 ± 2.82 A | 90.52 ± 1.85 A |
| 200 | – | 88.49 ± 3.29 A | 89.58 ± 1.75 A | 88.73 ± 1.47 A | 89.57 ± 1.75 A | 88.42 ± 2.48 A |

(C) Orange peels

| | | | | | | |
|---|---|---|---|---|---|---|
| 25 | 78.32 ± 4.21 A | – | – | – | – | – |
| 100 | – | 76.92 ± 2.19 A | 79.01 ± 3.11 A | 78.24 ± 1.88 A | 77.59 ± 2.44 A | 78.89 ± 3.62 A |
| 125 | – | 77.11 ± 3.10 A | 78.22 ± 2.68 A | 78.33 ± 1.94 A | 78.12 ± 1.31 A | 77.90 ± 2.19 A |
| 150 | – | 77.99 ± 3.11 A | 77.21 ± 3.19 A | 78.32 ± 2.84 A | 78.04 ± 2.38 A | 79.12 ± 2.65 A |
| 175 | – | 78.26 ± 4.08 A | 77.42 ± 2.08 A | 78.92 ± 3.18 A | 77.74 ± 2.22 A | 79.21 ± 1.95 A |
| 200 | – | 77.97 ± 1.22 A | 78.92 ± 1.01 A | 76.97 ± 3.02 A | 77.39 ± 1.74 A | 77.65 ± 2.18 A |

Values are means ± standard deviations from three replications.
Means followed by the same letter in a data series (column) are not significantly different ($P > .05$).

**Table 5**
Color analysis of steam treated cherry tomatoes at 4 °C during storage days.

| | Storage times (days) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 1 | 3 | 5 | 7 | 9 |
| L* | | | | | | |
| Untreated | 37.97 ± 1.55 Aa | 37.97 ± 1.55 Aa | 36.19 ± 1.11 Aa | 35.13 ± 2.19 Aa | 38.91 ± 3.01 Aa | 36.29 ± 1.22 Aa |
| 100 °C, 3 s | 38.03 ± 1.73 Aa | 36.19 ± 1.84 Aa | 37.21 ± 1.86 Aa | 36.22 ± 1.30 Aa | 36.21 ± 2.08 Aa | 37.79 ± 2.42 Aa |
| 200 °C, 3 s | 37.65 ± 0.66 Aa | 37.26 ± 1.82 Aa | 36.47 ± 1.19 Aa | 36.84 ± 1.49 Aa | 37.44 ± 1.21 Aa | 36.26 ± 1.59 Aa |
| a* | | | | | | |
| | 0 | 1 | 3 | 5 | 7 | 9 |
| Untreated | 15.82 ± 0.87 Aa | 15.82 ± 0.87 Aa | 15.85 ± 0.42 Aa | 16.23 ± 0.49 Aa | 16.04 ± 0.29 Aa | 15.70 ± 0.52 Aa |
| 100 °C, 3 s | 15.32 ± 0.67 Aa | 15.19 ± 0.12 Aa | 15.30 ± 0.28 Aa | 16.02 ± 0.73 Aa | 16.02 ± 0.24 Aa | 15.55 ± 0.54 Aa |
| 200 °C, 3 s | 16.51 ± 0.39 Aa | 15.22 ± 0.39 Aa | 15.68 ± 0.17 Aa | 15.81 ± 0.40 Aa | 16.87 ± 1.04 Aa | 16.04 ± 1.19 Aa |
| b* | | | | | | |
| | 0 | 1 | 3 | 5 | 7 | 9 |
| Untreated | 18.00 ± 0.37 Aa | 18.00 ± 0.37 Aa | 18.32 ± 0.41 Aa | 17.97 ± 0.42 Aa | 18.19 ± 0.52 Aa | 18.12 ± 0.43 Aa |
| 100 °C, 3 s | 18.04 ± 0.12 Aa | 18.22 ± 0.09 Aa | 18.05 ± 0.61 Aa | 18.08 ± 0.84 Aa | 17.97 ± 0.22 Aa | 18.29 ± 0.52 Aa |
| 200 °C, 3 s | 18.88 ± 0.63 Aa | 18.52 ± 0.21 Aa | 18.33 ± 0.55 Aa | 18.24 ± 0.27 Aa | 18.08 ± 0.36 Aa | 18.75 ± 0.61 Aa |

Values are means ± standard deviations from three replications.
Means with the same uppercase letter in the same column are not significantly different ($P < .05$).
Means with the same lowercase letter in the same row are not significantly different ($P < .05$).

and orange peels was 21.02, 48.90, and 28.29 mg/100 g, respectively. SS and SHS treated cherry tomatoes, orange pulp, and orange peels had a vitamin C content ranging from 18.89 to 23.17, 43.79 to 53.99, and 21.49 to 29.87 mg/100 g, respectively (Table 3). There were no statistically significant differences in vitamin C content between untreated and treated samples ($P > .05$) except orange pulp and peels steam treated for 20 and 30 s, respectively.

Antioxidant capacities of cherry tomatoes, orange pulp, and orange peels were measured as free radical-scavenging capacity in a DPPH· model. Fresh untreated cherry tomatoes, orange pulp, and orange peels exhibited 86.6, 89.4, and 78.3% inhibition, respectively (Table 4). Antioxidant capacity of SS and SHS treated cherry tomatoes, orange pulp, and orange peels were 85.5 to 88.6, 88 to 90.5, and 76.9 to 79.2% inhibition of DPPH·, respectively, with non-significant differences

**Table 6**
Maximum load values for texture of steam treated cherry tomatoes at 4 °C during storage days.

| | Storage times (days) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 1 | 3 | 5 | 7 | 9 |
| Untreated | 16.52 ± 1.43 Aa | 15.87 ± 0.53 Aa | 14.71 ± 1.77 Aa | 13.84 ± 1.23 Ba | 13.32 ± 1.30 Ba | 11.53 ± 0.57 Ca |
| 100 °C, 3 s | 16.43 ± 0.92 Aa | 16.47 ± 0.62 Aa | 14.34 ± 1.07 Aa | 13.89 ± 1.36 Ba | 12.76 ± 0.50 Ba | 11.42 ± 0.76 Ca |
| 200 °C, 3 s | 16.43 ± 0.77 Aa | 15.79 ± 1.30 Aa | 15.23 ± 1.88 Aa | 13.27 ± 0.94 Ba | 12.35 ± 1.65 Ba | 11.56 ± 0.86 Ca |

Values are means ± standard deviations from three replications.
Means with the same uppercase letter in the same column are not significantly different ($P < .05$).
Means with the same lowercase letter in the same row are not significantly different ($P < .05$).

between SS treated and untreated products ($P > .05$).

## 4. Discussion

Heat treatments have been used as staple means for insect disinfestation, decay control, ripening delay, and maintaining fruit quality during storage (Lurie, 1998; McDonald, McCollum, & Baldwin, 1999). Part of this reason may be due to a growing demand to decrease the use of chemicals against pathogens and insects on fresh produce (Lurie, 1998). Various heating technologies including conventional methods such as hot water, vapor heat, and hot air as well as more advanced methods like far infrared heating and radio frequency heating have been evaluated (Birla, Wang, Tang, & Hallman, 2004; Lurie, 1998; Tanaka et al., 2007). Until now, most studies have only involved moderate heat treatment of fresh produce and there has been no published research describing inactivation of foodborne pathogens on fresh fruits and vegetables by high temperature treatment. We therefore investigated the effect of SS and SHS on inactivation of foodborne pathogens and quality changes to fresh produce occurring during short heating treatment.

However, traditional SS treatment times were required for bacteria to be fully inactivated on fresh fruit. In the present study, SS treatment at 100 °C for 3 s only attained 1.4–2.7 log reductions for the three pathogens on cherry tomatoes and SS at 100 °C for 20 s only achieved log reductions of 2.3–2.6 on oranges. On the other hand, we observed that SHS treatment at 200 °C for 3 s attained full inactivation of the three pathogens on cherry tomatoes without changing the tomato appearance at 4 °C for up to 9 days.

When steam condenses on a cooled surface, a continuous film of condensate is formed which creates a thermal barrier to the further flow of heat (Tanner, Pope, Potter, & West, 1968). SHS receives additional heat to raise its temperature above the saturation temperature at a constant pressure and is transformed into low-moisture steam (dry steam) (Cenkowski, Pronyk, Zmidzinska, & Muir, 2007). When SHS contacts a surface, condensation temporarily occurs and then the condensed water evaporates back into the SHS, since moisture content in the chamber is low (Iyota, Nishimura, Yoshida, & Nomura, 2001). On the other hand, surfaces treated with SS experience little or no evaporation due to moisture saturation inside the chamber. The continuous film of condensate can protect bacteria from heat treatment and increase thermal resistance. For this reason, the inactivation effects of SS and SHS treatment on the three pathogens on cherry tomatoes and oranges differ due to the condensation film resulting from SS treatment.

Although fresh fruits and vegetables are sensitive to heat treatment, high temperature short time SHS treatment at 200 °C for 3 s and 20 s did not influence the quality of cherry tomatoes and oranges, respectively. To date, no research has been published dealing with the effect of high temperature short time SHS heat treatment of fresh fruits and vegetables.

In the present study, the results indicate that increasing temperature up to 200 °C for SHS treatment promotes the inactivation efficacy on cherry tomatoes and oranges compared to SS treatment. During storage, the populations of three pathogens treated with SS and SHS decreased. These results may be attributed to the higher level of injury to cells induced by extremely high heating temperatures rather than the availability of nutrients on the fruit surface and changes in water activity during storage (Chun, Kim, & Song, 2010). These results clearly suggest that the superheated steam treatment can be used as a heating technique for decreasing foodborne pathogen populations on cherry tomatoes and oranges and for enhancing microbial safety during storage without compromising sensory quality.

Up till the present time, many studies have demonstrated that commonly used conventional sanitizers only have a limited effect in reducing populations of pathogenic bacteria on fruits and vegetables. Lang, Harris, and Beuchat (2004) observed that populations of L. *monocytogenes*, *Salmonella* spp., and *E. coli* O157:H7 on lettuce were reduced by 1.1–1.8 log CFU/lettuce sample when treated with 200 μg/mL chlorine. Lee and Baek (2008) noticed that sodium hypochlorite treatment (100 ppm) for 5 min reduced levels of *E. coli* O157:H7 by 1.1 log CFU/g. Neal et al. (2012) reported that 1 mg/L ozone treatment for 30 min reduced levels of *Salmonella* and *E. coli* O157:H7 on spinach leaves by 1.0 and 0.6 log CFU/g, respectively. The necessity to develop an alternative technology for sanitation of fresh fruits and vegetables while not concurrently producing quality deterioration has increased.

This research demonstrated that SHS treatment leads to effective inactivation of *E. coli* O157:H7, *S*. Typhimurium, and L. *monocytogenes* on cherry tomatoes and oranges, as well as preventing quality deterioration. As interest in thermal treatment of fresh fruits and vegetables has increased, SHS technology has shown itself to be a very promising alternative intervention for improving microbial safety of fruits and vegetables as demonstrated by the authors on cherry tomatoes and oranges, while simultaneously reducing processing time and expense. Before commercial applications can be considered work needs to be carried out on the effect of SHS technology on products with low levels of natural contamination with bacteria. Studies also have to look at optimization of steam flow and temperature distribution.

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