Journal of Food Protection, Vol. 78, No. 6, 2015, Pages 1098–1105
doi:10.4315/0362-028X.JFP-14-522

Inactivation of *Escherichia coli* O157:H7 on Orange Fruit Surfaces and in Juice Using Photocatalysis and High Hydrostatic Pressure

SUNGYUL YOO,[1] KASHIF GHAFOOR,[2] JEONG UN KIM,[1] SANGHUN KIM,[1] BORA JUNG,[1] DONG-UN LEE,[3] AND JIYONG PARK[1]*

[1]*Department of Biotechnology, Yonsei University, Seoul 120-749, Republic of Korea;* [2]*Department of Food Science and Nutrition, King Saud University, P.O. Box 2460, Riyadh 11451, Kingdom of Saudi Arabia; and* [3]*Department of Food Science and Technology, Chung-Ang University, Anseong 456-756, Republic of Korea*

MS 14-522: Received 5 November 2014/Accepted 16 January 2015

ABSTRACT

Nonpasteurized orange juice is manufactured by squeezing juice from fruit without peel removal. Fruit surfaces may carry pathogenic microorganisms that can contaminate squeezed juice. Titanium dioxide–UVC photocatalysis (TUVP), a nonthermal technique capable of microbial inactivation via generation of hydroxyl radicals, was used to decontaminate orange surfaces. Levels of spot-inoculated *Escherichia coli* O157:H7 (initial level of 7.0 log CFU/cm^2) on oranges (12 cm^2) were reduced by 4.3 log CFU/ml when treated with TUVP (17.2 mW/cm^2). Reductions of 1.5, 3.9, and 3.6 log CFU/ml were achieved using tap water, chlorine (200 ppm), and UVC alone (23.7 mW/cm^2), respectively. *E. coli* O157:H7 in juice from TUVP (17.2 mW/cm^2)–treated oranges was reduced by 1.7 log CFU/ml. After orange juice was treated with high hydrostatic pressure (HHP) at 400 MPa for 1 min without any prior fruit surface disinfection, the level of *E. coli* O157:H7 was reduced by 2.4 log CFU/ml. However, the *E. coli* O157:H7 level in juice was reduced by 4.7 log CFU/ml (to lower than the detection limit) when TUVP treatment of oranges was followed by HHP treatment of juice, indicating a synergistic inactivation effect. The inactivation kinetics of *E. coli* O157:H7 on orange surfaces followed a biphasic model. HHP treatment did not affect the pH, °Brix, or color of juice. However, the ascorbic acid concentration and pectinmethylesterase activity were reduced by 35.1 and 34.7%, respectively.

Orange juice is the most widely consumed fruit juice because of its high nutritional value and pleasant taste *(29)*. Commercial orange juice products are mostly manufactured by direct squeezing of juice without removing the fruit peel, which may result in cross-contamination of the final product with pathogens from the fruit surface *(1)*. Acidic juices (pH 4.6 or less) containing enteric bacterial pathogens, such as *Escherichia coli* O157:H7, *Cryptosporidium parvum,* and various serovars of *Salmonella,* have caused serious foodborne illnesses *(38)*. *E. coli* O157:H7 can grow rapidly on raw fruits and vegetables stored at 12°C or higher. A small *E. coli* O157:H7 population can cause infection and can develop acid resistance *(15)*. Zhao et al. *(46)* reported that *E. coli* O157:H7 in ciders (pH 3.6 to 4.0) survived for 10 to 31 days at 8°C and for 2 to 3 days at 25°C.

The U.S. Food and Drug Administration (FDA) *(38)* hazard analysis critical control point regulations for fruit juices require use of treatments capable of consistently achieving at least a 5-log reduction in levels of pertinent microorganisms, such as *E. coli* O157:H7. Citrus fruit surface sanitizing steps also can be used before juice extraction. Chemical sanitizers, such as chlorine (50 to 200 ppm for 1 to 2 min), are widely used as industrial food surface disinfectants *(2, 6)*. However, chlorine solutions may achieve only minor inactivation of microorganisms on fruit surfaces *(43),* and potential health hazards, formation of undesirable by-products, and wastewater disposal problems discourage the use of chlorine for food disinfection. Therefore, alternative food surface decontamination methods are needed for the food industry.

Thermal processes have been commonly used to ensure the microbial safety of fruit juices; however, heat treatments can cause nutrient deterioration *(42)*. Hence, minimally or nonthermally processed foods with an increased shelf life and better nutritional properties are needed. Titanium dioxide (TiO_2)–UV photocatalysis (TUVP) is a new nonthermal technique that can inactivate pathogens under aqueous conditions via generation of strong oxidizing agents from the TiO_2-treated surface in the presence of UV light *(18)*. TUVP is more effective for microbial inactivation than is UV light alone, and an increase in the TiO_2-treated surface area and use of UVC light in a TUVP reactor can improve the bactericidal efficiency because of generation of more free radicals and improved exposure of liquid samples at the reaction site *(10)*.

High hydrostatic pressure (HHP) is another promising nonthermal technology that has increased in popularity because it preserves the nutritional and sensory characteristics and improves the overall quality of foods *(14)*.

* Author for correspondence. Tel: +82-2-2123-2888; Fax: +82-2-362-7265; E-mail: foodpro@yonsei.ac.kr.

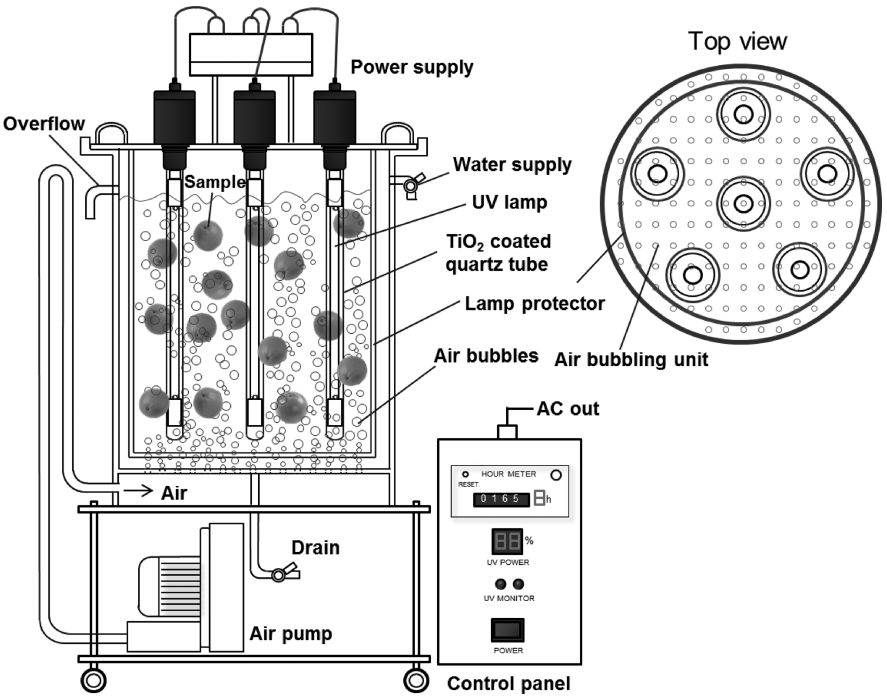

FIGURE 1. *Schematic diagrams of a lab-scale TiO_2-UVC photocatalysis (TUVP) reactor consisting of a 30-liter stainless steel vessel, an air pump, and six UV lamps (254 nm wavelength, 35 W, 25 mW/cm^2) in TiO_2-coated quartz tubes (24.5 mm outer diameter, 0.7 to 0.9 mm TiO_2 coating thickness). An air pump at the bottom of the reactor provided agitation during processing.*

However, information about the use of TUVP for fruit surface decontamination is lacking. Use of HHP treatment after TUVP-assisted fruit surface disinfection may result in synergistic pathogen inactivation and could be developed as alternatives or new processing methods for production of fruit juices.

E. coli strains differ widely in their resistance to pressure and other adverse conditions, including acidification, mild heat, and osmotic and oxidative stress *(1, 4, 30).* Therefore, *E. coli* O157:H7 was selected as a target bacterium in this study to evaluate the microbial inactivation effects of HHP and TUVP. Researchers have reported that the kinetics of microbial inactivation in foods by either thermal or nonthermal techniques are mostly not linear. Various nonlinear inactivation curves have been suggested following mathematical approaches, including a biphasic model *(8, 28, 45),* a Weibull model *(39),* and a log-linear shoulder-and-tail model *(13).* The objectives of this study were to (i) determine the effects of TUVP as a surface decontamination method for orange surfaces inoculated with *E. coli* O157:H7, (ii) determine an optimum pressure (HHP) in association with TUVP surface disinfection for complete inactivation of *E. coli* O157:H7 in freshly squeezed orange juice, (iii) determine the applicability of nonlinear models based on survival curves obtained after use of combined treatments, and (iv) study the effects of HHP on the quality attributes of orange juice.

MATERIALS AND METHODS

Microbial strain and test culture preparations. *E. coli* O157:H7 (NCTC 12079) was obtained from the National Collection of Type Cultures (London, UK). A stock culture was prepared by incubation of 1.0 ml of culture in 100 ml of nutrient broth at 38°C for 24 h with shaking. The cell suspension (30 ml) was centrifuged at 4,000 × *g* for 10 min, the supernatant was removed, and the cell pellet was resuspended in 5 ml of a 0.85% NaCl solution for use as the inoculum. Difco (BD, Franklin Lakes, NJ) culture medium and buffered peptone water were used.

Spot inoculation of oranges. Fresh oranges were purchased from a local market in Seoul, Korea, and stored at 4°C. The surface wax was washed off by immersion of fruit in hot water (70°C) for 30 s. Oranges were dried at room temperature, and a 12-cm^2 area of each orange (smooth part) was inoculated using drops of 10 or 100 μl of the *E. coli* O157:H7 inoculum to obtain a population of 7.0 log CFU/cm^2. Oranges were kept at room temperature for 1 h before surface decontamination treatments.

Surface decontamination of oranges. A TUVP reactor was customized (Fig. 1) according to a previous study *(19).* The reactor consisted of a stainless steel 30-liter capacity vessel, an air pump, and six UVC lamps (254 nm wavelength, 35 W, 25 mW/cm^2; Sankyo Denki, Tokyo, Japan) in TiO_2-coated quartz tubes (38 cm length, 24.5 mm outer diameter, TiO_2 coating thickness 0.7 to 0.9 mm; Taekyeong UV Co., Namyangju, Korea). An air pump at the bottom of the reactor was used to produce agitation during treatment of the orange fruit. UVC lamps fitted into TiO_2-coated quartz tubes were placed in the TUVP reactor cap. UVC lamps were fitted into quartz tubes for UVC treatment alone. The assemblies were submerged in water inside the reactor. Within the same reactor, a water bubble (WB) treatment was carried out as a control by disconnecting the UVC lamp TiO_2 assembly. A chlorine solution (200 ppm, pH 9.0) was prepared using a 12% hypochlorite solution (Yakuri Pure Chemicals Co., Kyoto, Japan) and, similar to the WB treatment, was used for treatment of oranges in the same reactor without photocatalysis. Inoculated oranges were immersed in a batch containing 28 liters of tap water, and TUVP, UVC, and WB treatments were applied. Samples were treated for 0, 1, 3, 5, 10, and 20 min, after which microbial levels were determined. Treatment with the chlorine solution followed an immediate neutralizing step (washing in tap water for 1 min).

Immersion of oranges in the inoculum for juice extraction. Oranges were immersed in the inoculum for juice extraction, based on the method of Bagci and Temiz *(2),* for 30 s and then

drained for 1 h to remove excess inoculum before juice extraction. Seven oranges per treatment (approximately 2 kg) were squeezed with a commercial hand type juice extractor (G101, Lemonice Co., Seoul, Korea). Extractors were autoclaved before extraction. Juice samples were either evaluated for microbial counts or subjected to HHP treatment.

HHP treatment of orange juice. Orange juice was treated with HHP at ambient temperatures in a hydrostatic pressurization unit (HHP-600, Baotou Kefa Co., Inner Mongolia, People's Republic of China) with a 5.0-liter capacity. The pressure was increased at a rate of 2 MPa/s and the decompression time was 3 to 4 s. Distilled water was used as the pressure transmission fluid. The pressure holding time in this study did not include the pressure increase and depressurization times. Samples (TUVP, UVC, chlorine, and WB treatments) were packed in flexible polyethylene terephthalate pouches (10 by 15 cm) and heat sealed with no headspace. Packed samples were loaded into the vessel and pressurized at 300 to 500 MPa for 1 min at 25°C.

pH, °Brix, and color analysis. The pH of orange juice was monitored with a pH meter (520A, Thermo Orion, MA), and °Brix was measured at 25°C with an automatic refractometer (Smart-1, Atago Co., Tokyo, Japan). Color was analyzed with a chromameter equipped with the Hunter color system (Konica CR-400, Minolta Sensing Inc., Tokyo, Japan). The chromameter was calibrated with a white tile ($Y = 92.3$, $x = 0.3156$, $y = 0.3433$). Color values were expressed as L* (lightness or brightness/darkness), a* (redness), and b* (yellowness). The total color change (ΔE) was determined using equation 1 to monitor the magnitude of the color change before (L_o, a_o, and b_o) and after (L*, a*, and b*) HHP treatment:

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

The value of ΔE was categorized as not noticeable (0 to 0.5), slightly noticeable (0.5 to 1.5), noticeable (1.5 to 3.0), easily visible (3.0 to 6.0), and greatly different (6.0 to 12.0) *(41)*.

Determination of ascorbic acid retention. Ascorbic acid retention (AAR) was monitored using a high-performance liquid chromatography method *(23)* with some modification. Freshly squeezed orange juice (5 ml) was placed in a 50-ml centrifuge tube containing 20 ml of 4.5% metaphosphoric acid. The sample was centrifuged for 15 min at 1,640 × *g*, and 0.5 ml of the supernatant was placed in a volumetric flask and mixed with 4.5% metaphosphoric acid to make up the volume to 10 ml. This solution was then filtered with a 0.45-μm-pore-size nylon filter (Fisher Scientific, Loughborough, UK). The chromatographic system (Dionex Corporation, Sunnyvale, CA) consisted of an autosampler, pump, column oven, UV detector, and a reversed phase column (4.6 by 250 mm; Shisedo, Tokyo, Japan). The solvent was 2% KH_2PO_4 (pH 2.4) at a flow rate of 1.0 ml/min. The injection volume was 20 μl for both the standard and the sample solutions. The AAR value of treated samples was calculated using equation 2:

$$\text{AAR } (\%) = \frac{\text{AA of treated sample}}{\text{AA of untreated sample}} \times 100 \quad (2)$$

Pectinmethylesterase activity analysis. The pectinmethylesterase (PME) activity in orange juice was determined according to previously described methods *(12, 20)*. Release of acid during pectin hydrolysis was measured as a function of time at a pH of 7.75 and 20 ± 1°C. The reaction mixture consisted of 5 ml of orange juice and 100 ml of a 1% (wt/vol) citrus pectin (Sigma-Aldrich, St. Louis, MO) solution in 0.15 M NaCl. Reaction components were mixed, and the pH was quickly adjusted and maintained at 7.75 by constant addition of 0.01 M NaOH. The enzyme activity was directly related to the amount of NaOH used per minute. One PME unit (PMEU) was defined as the amount of 0.01 M NaOH needed per minute to constantly maintain the initial pH (equation 3) *(12)*. The PME activity of each sample was measured in triplicate.

$$\text{PMEU} = \frac{\text{ml of 0.01 M NaOH}}{\text{(5 ml of sample) (minutes)}} \quad (3)$$

The relative PME activity was calculated according to equation 4 *(20)*:

$$\text{Relative PME activity } (\%) = \frac{\text{PMEU of treated juice}}{\text{PMEU of untreated juice}} \times 100 \quad (4)$$

Microbiological analysis. *E. coli* O157:H7 (initial level of 7.0 log CFU/cm^2) spot inoculated on orange surfaces (12 cm^2 area) was enumerated based on a modified method *(29)*. A piece of the inoculated area, excised to 4 mm depth, was placed in a stomacher filter bag (Interscience, St. Nom, France) with 50 ml of 1% buffered peptone water and mixed with a stomacher (MIX 2, AES Laboratories, Combourg, France) for 2 min. The filtered sample was immediately used for microbial analysis.

The total plate count method was used to enumerate viable microbial cells in orange juice. Before and after inactivation treatments, 1.0-ml juice samples were serially diluted in a sterile 0.85% NaCl solution, and 1.0 ml of each solution was pour plated onto triplicate plates of nutrient agar. *E. coli* O157:H7 colonies were counted after incubation at 37°C for 24 h. The log N/N_0 was calculated to determine inactivation effects, where N_0 is the initial *E. coli* O157:H7 count (4.5 log CFU/ml) before treatment and N is the count of viable *E. coli* O157:H7 in juice samples obtained from surface-disinfected oranges.

Mathematical models and assessment. Survival curves for surface decontamination treatments were obtained based on plots of the logarithm of the survival fraction against the treatment time. Fitting of survival curves and calculation of resistance parameters were accomplishing using the GInaFiT inactivation model fitting tool *(13)*. Because survival curves in this study did not show shoulders or tails, the biphasic and Weibull models were used for survival curve fitting.

The biphasic model is defined as:

$$\log N = \log N_0 + \log\left[fe^{-k_{\max 1}t} + (1-f)e^{-k_{\max 2}t}\right]$$

where f is the fraction of the sensitive population, $(1 - f)$ is the fraction of the resistant population, $k_{\max 1}$ and $k_{\max 2}$ are the inactivation rates for these two populations, and t is the treatment time (minutes) *(8, 22)*.

The Weibull model is defined as:

$$\log N = \log N_0 - bt^n$$

where b is a scale factor, n is a shape factor, and t is the treatment time (minutes) *(31)*.

Statistical analysis. All measurements were done in triplicate, and results were expressed as the mean ± the standard deviation. Data were analyzed using the one-way analysis of variance function of the Statistical Package for the Social Sciences software (SPSS version 21, IBM Corporation, Armonk, NY). The Student-Newman-Keuls test was also used for statistical analysis, and the confidence level for statistical significance was based on a P value of 0.05.

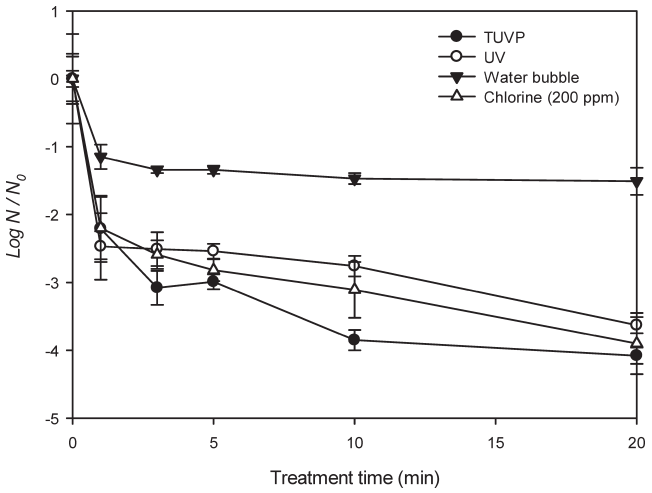

FIGURE 2. *Effects of water bubble, chlorine (200 ppm), UVC, and TiO_2-UV photocatalysis (TUVP) treatments for inactivation of* E. coli *O157:H7 inoculated onto orange surfaces. (N_0 = 7.0 log CFU/cm^2).*

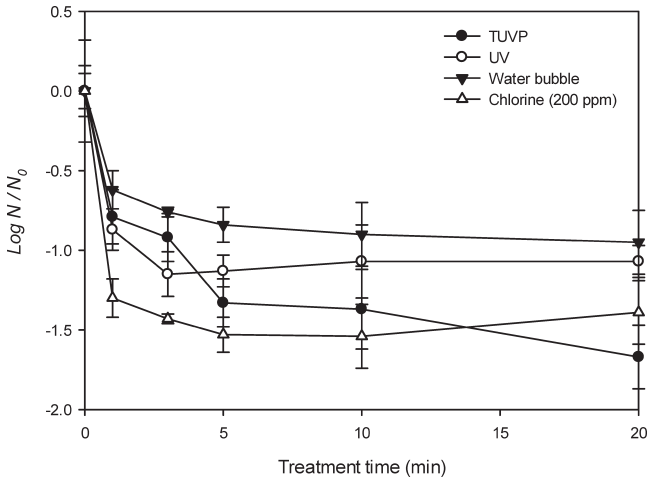

FIGURE 3. *Effects of water bubble, chlorine (200 ppm), UVC, and TiO_2-UV photocatalysis (TUVP) treatments for inactivation of* E. coli *O157:H7 in freshly squeezed juice made from inoculated oranges (N_0 = 4.5 log CFU/ml).*

RESULTS AND DISCUSSION

Inactivation effects of TUVP, UVC, chlorine, and WB on *E. coli* O157:H7 inoculated onto orange surfaces and into juice. The effects of surface decontamination treatments are shown in Figure 2. The average initial population of *E. coli* O157:H7 spot inoculated onto orange surfaces was 7.0 log CFU/cm^2. The inactivation of *E. coli* O157:H7 by TUVP (17.2 mW/cm^2), UVC (23.7 mW/cm^2), chlorine (200 ppm), and WB treatments for 20 min was in the order of TUVP (4.08 log CFU/cm^2) > chlorine (3.90 log CFU/cm^2) > UVC (3.63 log CFU/cm^2) > WB (1.51 log CFU/cm^2). Although the TUVP dose was lower than the UVC dose, the TUVP inactivation efficiency was higher, probably because of the radicals generated after excitation of TiO_2 particles. The TiO_2 particles were coated in a thin layer on the surface of quartz tubes (UVC source) and generated free radicals after UV penetration (photocatalysis), resulting in enhanced disinfection effects *(18).* TUVP treatment reduced the initial *E. coli* O157:H7 counts (7.0 log CFU/cm^2) on orange surfaces to 2.92 log CFU/cm^2, whereas UVC, chlorine, and WB treatments reduced counts to 3.37, 3.10, and 5.49 log CFU/cm^2, respectively. The disinfection effects of TUVP treatment after 10 min were significantly different ($P < 0.05$) from the effects of the other treatments.

In a previous study *(2),* a boiling water treatment for 30 s caused a 3.37-log reduction of *E. coli* O157:H7 inoculated onto orange surfaces. A similar *E. coli* O157:H7 inactivation was achieved with a 200 ppm of chlorine solution, which is a commonly used industrial food disinfectant. The higher efficiency of TUVP is related to induced destruction of the cytoplasmic membrane, DNA damage, and rupture of the internal organization of *E. coli, Listeria monocytogenes,* and *Salmonella* Typhimurium cells in liquid cultures *(18),* leading to leakage of cytoplasmic contents and cell death *(21).* TUVP results in generation of the reactive oxygen species OH, O_2^-, and H_2O_2. When TiO_2 is exposed to UV light, positively charged holes are generated in the valance band of TiO_2 molecules. Hydroxyl radicals are formed when these holes react with adsorbed H_2O or OH^- at the TiO_2 surface. Electrons, generated in a conduction band, react with oxygen to form hydroxyl radicals. These reactive oxygen species can inactivate enzymes and cause acute oxidative stress in bacterial cellular components *(16).* Hence, the higher efficiency of TUVP can be attributed to multiple mechanisms affecting structures and functions of microbial cells.

Oranges that were surface inoculated with *E. coli* O157:H7 were disinfected by various methods, the fruits were squeezed to obtain juice, and cross-contamination from fruit to juice was evaluated by determining the levels of *E. coli* O157:H7 in the juice. Juice was also obtained from untreated oranges as a control, and the *E. coli* O157:H7 level for this juice was approximately 4.5 log CFU/ml. Thus, *E. coli* O157:H7 present on the orange surface can be transferred to juice, highlighting the importance of surface disinfection before juice processing and consumption. For juice obtained from oranges in the TUVP, UVC, chlorine, and WB treatment groups, 1.67-, 0.93-, 1.39-, and 0.95-log reductions in *E. coli* O157:H7 counts, respectively, were found after 20 min of treatment (Fig. 3). In a previous study *(2),* 0.79-, 3.05-, 2.22-, and 2.71-log reductions of *E. coli* were reported for juice from oranges treated with distilled water (2 min), boiling water (0.5 min), chlorine (200 ppm, 8 min), and H_2O_2 (5%, 2 min), respectively. Thus, any pathogenic microbe on the fruit surface is a potential hazard for human health, and fruit surface disinfection alone cannot reduce the numbers of pathogens to acceptable levels in the final fruit products. Hence, a multipronged strategy is required to ensure that fruits and fruit products are safe from foodborne pathogens. TUVP is a good alternative fruit surface decontamination method for inactivation of *E. coli* O157:H7. However, the 5-log reduction recommended by the FDA *(38)* was not achieved with any treatment. Therefore, additional treatments may be required to ensure the microbial safety of orange products.

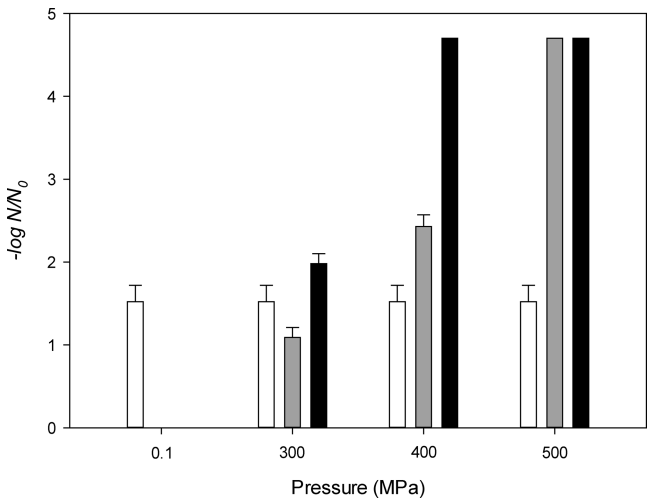

FIGURE 4. *Inactivation of* E. coli *O157:H7 in freshly squeezed orange juice after TiO_2-UVC photocatalysis (TUVP) and TUVP-assisted orange surface disinfection alone (open bars), HHP treatment of juice alone (shaded bars), and a combination of both TUVP (orange surface) and HHP (juice) treatments (solid bars).*

Effects of HHP on inactivation of *E. coli* O157:H7 in orange juice. TUVP treatment of oranges for 20 min resulted in a maximum inactivation of *E. coli*. O157:H7 on orange surfaces and in juice. Juice from TUVP-treated or untreated oranges was subjected to HHP treatment (0.1 to 500 MPa) for 1 min. Results of HHP-assisted inactivation of *E. coli* O157:H7 are shown in Figure 4 (from initial counts of 4.50 log CFU/ml). Juice obtained from TUVP-treated oranges (17.2 mW/cm^2, 20 min) was processed with HHP at 400 MPa, and complete inactivation of *E. coli* O157:H7 was achieved. Disinfection achieved with the combined TUVP plus HHP (400 MPa) treatment was significantly more effective ($P < 0.05$) than that achieved with TUVP and HHP treatments alone. TUVP-assisted surface decontamination of oranges and a subsequent 1-min HHP treatment at 400 MPa had a synergistic disinfection effect, resulting in complete inactivation of *E. coli*. O157:H7 in orange juice. Use of the combined treatment may allow a lower pressure to be used in the HHP treatment to obtain the same juice disinfection effect and to ensure the safety of fruit before juice extraction *(9)*.

In previous reports, fruit juice samples obtained from fruits not subjected to surface decontamination treatments had high levels of barotolerant microorganisms. A-500 MPa HHP treatment achieved a 5-log reduction of *E. coli* O157 C9490 (a pressure-resistant strain) in apple (pH 3.5) and tomato (pH 4.1) juices but only a 1- to 2-log reduction in orange juice (pH 3.8) *(17)*. Similar reduction was achieved for *E. coli* ATCC 11775 at a pressure as low as 200 MPa *(17)*. Linton et al. *(24)* reported that *E. coli* O157:H7 inoculated into orange juice (pH 3.9) was reduced by 4 log CFU/ml after treatment at 550 MPa for 1 min. Hence, *E. coli* O157:H7 is a pressure-resistant pathogen *(30)*, and a combination of pulsed electric field, UVC, mild heat, and chemicals with HHP can achieve more efficient inactivation in foods *(9, 24, 26, 44)*.

E. coli O157:H7 cells were subjected to UV radiation and photocatalytic oxidation when oranges were treated using TUVP. UV light passing through TiO_2-coated quartz tubes penetrates microbial cell membranes and disrupts DNA *(18, 35)*. Photocatalysis oxidizes constituents of microbial cells, especially cell membranes *(25)*. Disruption of microbial DNA and membranes leads to cellular stress, organelle damage, and cell death. Chai et al. *(9)* observed that a combination of TUVP and HHP resulted in synergistic inactivation of *Bacillus cereus* endospores in a green *Angelica keiskei* juice. TUVP treatment of fruits was important for ensuring maximum inactivation effects of HHP in juice. Thus, sublethal injury to *E. coli* O157:H7 cells treated with TUVP leads to enhanced synergistic inactivation when combined with HHP treatment.

Modeling the inactivation kinetics of *E. coli* O157:H7 on the fruit surface. The inactivation kinetics of surface decontamination of oranges inoculated with *E. coli* O157:H7 were fitted to the biphasic and Weibull models. The Weibull model was not suitable as indicated by lower (<0.9) R^2 values (data not shown). Therefore, survival curves of surface-inoculated *E. coli* O157:H7 cells after treatments for 1, 3, 5, 10, and 20 min were fitted to the biphasic model (Table 1). Survival curves of *E. coli* O157:H7 on the orange surface and in freshly squeezed juice were investigated. Results were similar to reductions

TABLE 1. *Parameters and assessment of the biphasic model*

Sample	Treatment	Model parameters[a]			R^2	MSE
		f	k_{max1}	k_{max2}		
Surface-inoculated oranges, 7.0 log CFU/cm^2	Water bubble	0.9514 ± 0.0062 A	3.71 ± 0.35 D	0.02 ± 0.01 B	0.9978	0.0419
	Chlorine	0.9959 ± 0.0006 A	5.84 ± 0.29 B	0.17 ± 0.01 A	0.9995	0.0481
	UVC	0.9963 ± 0.0032 A	8.91 ± 1.86 A	0.13 ± 0.05 A	0.9789	0.2839
	TUVP	0.9987 ± 0.0011 A	5.29 ± 1.03 C	0.15 ± 0.05 A	0.9856	0.2816
Orange juice from surface-inoculated oranges, 4.5 log CFU/ml	Water bubble	0.8956 ± 0.0547 B	2.41 ± 0.35 C	0.02 ± 0.01 A	0.9949	0.0397
	Chlorine	0.9663 ± 0.0094 A	4.07 ± 0.76 A	0.00 ± 0.02 A	0.9907	0.0907
	UVC	0.9215 ± 0.0123 A	2.80 ± 0.54 B	0.00 ± 0.01 A	0.9347	0.0510
	TUVP	0.8956 ± 0.0547 B	2.56 ± 1.13 C	0.08 ± 0.03 A	0.9666	0.1707

[a] Values are mean ± standard deviation. Within a column, means followed by different letters are significantly different ($P < 0.05$). f, fraction of the sensitive population; k_{max1}, inactivation rate of sensitive population; k_{max2}, inactivation rate of resistant population; R^2, regression coefficient; MSE, mean square error.

of total plate counts in nonhomogenized cucumber juice *(45)* and *E. coli* inactivation in liquid whole egg *(22)* after HHP treatment. The inactivation kinetics of *E. coli* after exposure to mild heat and UVA also followed a true biphasic model *(5)*.

Differences in f values of the four orange surface disinfection methods were not significant ($P > 0.05$), indicating that the fraction of the sensitive population was not influenced by these treatments (Table 1). However, the k_{max1} values for the disinfection of *E. coli* O157:H7 cells inoculated onto orange surfaces were significantly different ($P < 0.05$) (Table 1). UVC treatment had the highest k_{max1} value with the fastest sensitive population inactivation rate. Dose intensities of UV lamps used for UVC and TUVP treatments were 23.7 and 17.2 mW/cm^2, respectively. The initial level of *E. coli* O157:H7 exposure was higher for UVC than for TUVP. TiO_2 particles coating the lamp as a thin layer blocked a portion of the UV light, which resulted in a lower initial inoculum level. Although the UVC treatment had a higher k_{max1} value, TUVP achieved a greater *E. coli* O157:H7 reduction, which was attributed to the production of reactive oxygen species from photocatalysis during the treatment time. The k_{max1} values for fresh orange juice also were not significantly different except for the chlorine treatment (Table 1). These values may be incorrect because other microorganisms may exist in fruit pulp and a low pH may influence growth of *E. coli* O157:H7, which moved into the juice via cross-contamination from the surface. The k_{max2} values for inactivation of *E. coli* O157:H7 on both orange surfaces and juice were not significantly different, indicating that inactivation of the resistant population was not affected by the treatments.

Effects of HHP treatment on physicochemical properties of orange juice. Evaluation of orange juice quality and nutritional properties after an optimum nonthermal treatment is important because consumers demand high-quality products. The orange surface TUVP treatment (17.2 mW/cm^2) for 20 min followed by juice extraction and HHP treatment of juice (400 MPa) resulted in complete inactivation of *E. coli* O157:H7 in the juice. HHP treatment of juice at 500 MPa also resulted in complete inactivation of *E. coli* O157:H7 without previous surface decontamination. Fruit surface decontamination treatments (such as TUVP) probably do not influence the quality of orange juice. Hence, the effects of TUVP on juice quality were not investigated in the present study. However, application of TUVP fruit surface treatment alone cannot ensure microbial safety of juice obtained from surface-disinfected fruits and may require further treatment. Orange juice was evaluated for the physicochemical properties of pH, °Brix, color, ascorbic acid concentration, and PME activity after HHP (500 MPa) (Table 2). Compared with nontreated (control) freshly squeezed orange juice, there were no significant differences ($P > 0.05$) in pH, °Brix, L*, a*, and b* values of HHP-treated and nontreated orange juices. The total color difference (ΔE), which indicates the magnitude of color change *(3)* due to HHP, between treated sample and control samples was 0.37 (not noticeable) *(40)*. Nonsignificant changes in pH, solids,

TABLE 2. *Physicochemical properties (pH, °Brix, color values, pectin methyl esterase [PME] activity, and ascorbic acid concentration) of untreated (control) and HHP (500 MPa)–treated freshly squeezed orange juice*[a]

Orange juice sample	pH	°Brix	L*	a*	b*	ΔE	Relative ascorbic acid (%)	PME	Relative PME activity (%)
Untreated	3.58 ± 0.22	14.72 ± 0.44	23.81 ± 0.34	−3.66 ± 0.01	8.76 ± 0.19			0.31 ± 0.035	
HHP treated	3.57 ± 0.23	14.72 ± 0.43	23.26 ± 0.41	−3.59 ± 0.32	8.40 ± 0.41	0.37 ± 0.40	35.10 ± 5.72	0.21 ± 0.019	34.70 ± 7.48

[a] Values are mean ± standard deviation ($n \geq 3$). L*, lightness; a*, redness; b*, yellowness; ΔE, total color change.

and color characteristics of orange juice after HHP treatment have been previously reported *(7, 11)*.

The initial ascorbic acid concentration of juice decreased by 35.10% after the 500 MPa HHP treatment. Gayán et al. *(12)* reported that even a mild heat treatment (55°C) combined with UV light (23.72 J/ml) resulted in a reduction in the ascorbic acid concentration of orange juice, and this loss was mainly attributed to UV light. Uckoo et al. *(37)* also observed that ascorbic acid concentration in HHP-treated (400 MPa, 3 min) grapefruit juice decreased; however, this decrease was less than that observed for thermally treated (85°C, 45 s) juice.

Polydera et al. *(32)* reported a slower ascorbic acid loss rate when orange juice was treated with high pressure in comparison with high heat. Processing and storage can result in loss of ascorbic acid in orange juice. However, the loss due to HHP is lower than that due to heat treatments. No significant loss of ascorbic acid in HHP-treated orange juice was observed after storage for 21 days at 4°C compared with controls. Results of the present study agree with those of Bull et al. *(7),* who reported that the ascorbic acid concentration decreased in HHP-treated orange juice because of oxidation attributed to oxygen diffusion through the packaging and anaerobic decomposition of the ascorbic acid. However, Bull et al. also found that the decrease in the ascorbic acid concentration induced of orange juice by HHP was less than that induced by heat treatment. More studies are required to develop processing strategies for prevention of ascorbic acid loss in orange juice.

A major problem associated with orange juice quality deterioration is cloud loss accompanied by gelation of juice concentrates, primarily attributed to PME activity *(33)*. HHP treatment (500 MPa) resulted in a 34.7% reduction in PME activity in orange juice. Sampedro et al. *(34)* found no decrease in PME activity after a 450-MPa treatment of a milk–orange juice beverage. Thus, PME inactivation may also depend on the product matrix that carries this enzyme, and higher pressures may be required. Similarly, other researchers *(27, 36)* also reported that pressures lower than 400 MPa resulted in no change in PME activity in orange juice, and a combined treatment involving heat (40 to 50°C) and high pressure (600 to 700 MPa) was required for inhibition of PME.

Inactivation of PME in orange juice may also depend on the juice composition, which can differ according to the orange cultivar. Bull et al. *(7)* reported that HHP treatment at 600 MPa for 1 min did not result in significant PME inactivation in Valencia orange juice, whereas significant reduction in activity was observed in Navel orange juice. In the same study, a heat treatment of 65 to 85°C for 1 min significantly reduced PME activity. Complete PME inactivation (99%) in common industrial heat-processed orange juice is achieved by heating at 90°C for 1 min, which may be detrimental to the nutritional and sensory attributes of juice *(33)*. To achieve the same level of inactivation using nonthermal technologies, further studies are required. An effective approach may be a combined treatment involving use of mild heat and HHP treatment to achieve substantial or complete PME inactivation.

TUVP disinfection of orange surfaces is an innovative technique for inactivation of pathogenic bacteria. *E. coli* O157:H7 inactivation on orange surfaces was higher than that achieved with other routinely used food surface disinfection methods evaluated in this study. TUVP surface disinfection before HHP treatment of freshly squeezed orange juice increased the inactivation of *E. coli* O157:H7 in the final juice product. TUVP treatment reduced *E. coli* O157:H7 levels from 7.00 to 4.08 log CFU/ml on orange surfaces. TUVP treatment of oranges resulted in larger reductions of *E. coli* O157:H7 in fresh juice from cross-contamination than did other methods. However, the desired 5-log inactivation of *E. coli* O157:H7 was not achieved in juice. Therefore, a TUVP treatment (17.2 mW/cm^2, 20 min) of oranges was followed by HHP (400 MPa) processing of juice, which resulted in nearly a 5-log reduction of *E. coli* O157:H7 levels, as required by the FDA *(38)*. Other combination treatments of HHP plus pulsed electric fields, UVC, mild heating, and chemicals have similarly reduced targeted microorganisms in fruit and vegetable juices *(9, 24, 26, 44)*. The inactivation kinetics of TUVP treatment of *E. coli* O157:H7 inoculated onto oranges fit a biphasic model, and HHP treatment (500 MPa) did not change pH, °Brix, or color attributes. PEM activity was decreased by 34.7% in HHP-treated juice. Strategies for prevention of ascorbic acid loss in orange juice (35.1% loss after 500-MPa HHP treatments) are needed when a combined TUVP and HHP process is used to ensure the safety and quality of oranges and orange products. Effects of various combination treatments on other quality attributes of orange juice also should be studied.

ACKNOWLEDGMENT

This research was supported by the High Value-Added Food Technology Development Program (Ministry of Agriculture, Food and Rural Affairs, Republic of Korea).

REFERENCES

1. Alpas, H., N. Kalchayanand, F. Bozoglu, A. Sikes, C. Dunne, and B. Ray. 1999. Variation in resistance to hydrostatic pressure among strains of food-borne pathogens. *Appl. Environ. Microbiol.* 65:4248–4251.
2. Bagci, U., and A. Temiz. 2011. Microbiological quality of fresh-squeezed orange juice and efficacy of fruit surface decontamination methods in microbiological quality. *J. Food Prot.* 74:1238–1244.
3. Barba, F. J., M. J. Esteve, and A. Frigola. 2010. Ascorbic acid is the only bioactive that is better preserved by high hydrostatic pressure than by thermal treatment of a vegetable beverage. *J. Agric. Food Chem.* 58:10070–10075.
4. Benito, A., G. Ventoura, M. Casadei, T. Robinson, and B. Mackey. 1999. Variation in resistance of natural isolates of *Escherichia coli* O157 to high hydrostatic pressure, mild heat, and other stresses. *Appl. Environ. Microbiol.* 65:1564–1569.
5. Berney, M., H. U. Weilenmann, J. Ihssen, C. Bassin, and T. Egli. 2006. Specific growth rate determines the sensitivity of *Escherichia coli* to thermal, UVA, and solar disinfection. *Appl. Environ. Microbiol.* 72:2586–2593.
6. Beuchat, L. R., and J. H. Ryu. 1997. Produce handling and processing practices. *Emerg. Infect. Dis.* 3:459–465.
7. Bull, M. K., K. Zerdin, E. Howe, D. Goicoechea, P. Paramanandhan, R. Stockman, J. Sellahewa, E. A. Szabo, R. L. Johnson, and C. M. Stewart. 2004. The effect of high pressure processing on the

microbial, physical and chemical properties of Valencia and Navel orange juice. *Innov. Food Sci. Emerg. Technol.* 5:135–149.
8. Cerf, O. 1977. A review—tailing of survival curves of bacterial spores. *J. Appl. Bacteriol.* 42:1–19.
9. Chai, C., J. Lee, Y. Lee, S. Na, and J. Park. 2014. A combination of TiO_2–UV photocatalysis and high hydrostatic pressure to inactivate *Bacillus cereus* in freshly squeezed *Angelica keiskei* juice. *LWT Food Sci. Technol.* 55:104–109.
10. Feng, M., K. Ghafoor, B. Seo, K. Yang, and J. Park. 2013. Effects of ultraviolet-C treatment in Teflon®-coil on microbial populations and physico-chemical characteristics of watermelon juice. *Innov. Food Sci. Emerg. Technol.* 19:133–139.
11. Fernandez, M., R. Rodrıguez, Y. Pico, and J. Manes. 2001. Liquid chromatographic–mass spectrometric determination of post-harvest fungicides in citrus fruits. *J. Chromatogr. A* 912:301–310.
12. Gayán, E., M. J. Serrano, S. Monfort, I. Álvarez, and S. Condón. 2012. Combining ultraviolet light and mild temperatures for the inactivation of *Escherichia coli* in orange juice. *J. Food Eng.* 113: 598–605.
13. Geeraerd, A. H., V. P. Valdramidis, and J. F. Van Impe. 2005. GInaFiT, a freeware tool to assess non-log-linear microbial survivor curves. *Int. J. Food Microbiol.* 102:95–105.
14. Ghafoor, K., S.-O. Kim, D.-U. Lee, K. Seong, and J. Park. 2012. Effects of high hydrostatic pressure on structure and colour of red ginseng (*Panax ginseng*). *J. Sci. Food Agric.* 92:2975–2982.
15. Harris, L. J., J. N. Farber, L. R. Beuchat, M. E. Parish, E. H. Suslow, E. H. Garrett, and F. F. Busta. 2003. Outbreaks associated with fresh produce—incidence, growth, and survival of pathogens in fresh and fresh-cut produce. *Compr. Rev. Food. Sci. Food Saf.* 2:78–141.
16. Ireland, J. C., P. Klostermann, E. W. Rice, and R. M. Clark. 1993. Inactivation of *Escherichia coli* by titanium dioxide photocatalytic oxidation. *Appl. Environ. Microbiol.* 59:1668–1670.
17. Jordan, S. L., C. Pascual, E. Bracey, and B. M. Mackey. 2001. Inactivation and injury of pressure-resistant strains of *Escherichia coli* O157 and *Listeria monocytogenes* in fruit juices. *J. Appl. Microbiol.* 91:463–469.
18. Kim, S., K. Ghafoor, J. Lee, M. Feng, J. Hong, D.-U. Lee, and J. Park. 2013. Bacterial inactivation in water, DNA strand breaking, and membrane damage induced by ultraviolet-assisted titanium dioxide photocatalysis. *Water Res.* 47:4403–4411.
19. Kim, Y., Y. Choi, S. Kim, J. Park, M. Chung, K. B. Song, I. Hwang, and K. Kwon. 2009. Disinfection of iceberg lettuce by titanium dioxide–UV photocatalytic reaction. *J. Food Prot.* 72:1916–1922.
20. Kimball, D. A. 2002. Procesado de cítricos. Espasa Calpe, Madrid.
21. Krishnamurthy, K., J. Tewari, J. Irudayaraj, and A. Demirci. 2010. Microscopic and spectroscopic evaluation of inactivation of *Staphylococcus aureus* by pulsed UV light and infrared heating. *Food Bioprocess Technol.* 3:93–104.
22. Lee, D.-U., V. Heinz, and D. Knorr. 2001. Biphasic inactivation kinetics of *Escherichia coli* in liquid whole egg by high hydrostatic pressure treatments. *Biotechnol. Progr.* 17:1020–1025.
23. Lee, H. S., and G. A. Coates. 1996. Vitamin C in frozen, fresh squeezed, unpasteurized, polyethylene-bottled orange juice: a storage study. *Food Chem.* 65:165–168.
24. Linton, M., J. M. McClements, and M. F. Patterson. 1999. Inactivation of *Escherichia coli* O157:H7 in orange juice using a combination of high pressure and mild heat. *J. Food Prot.* 62:277–279.
25. Maness, P. C., S. Smolinski, D. M. Blake, Z. Huang, E. J. Wolfrum, and W. A. Jacoby. 1999. Bactericidal activity of photocatalytic TiO_2 reaction: toward an understanding of its killing mechanism. *Appl. Environ. Microbiol.* 65:4094–4098.
26. Marx, G., A. Moody, and D. Bermudez-Aguirre. 2011. A comparative study on the structure of *Saccharomyces cerevisiae* under nonthermal technologies: high hydrostatic pressure, pulsed electric fields and thermo-sonication. *Int. J. Food Microbiol.* 151: 327–337.
27. Nienaber, U., and T. H. Shellhammer. 2001. High-pressure processing of orange juice: kinetics of pectinmethylesterase inactivation. *J. Food Sci.* 66:328–331.
28. Panagou, E. Z., C. C. Tassou, C. Manitsa, and C. Mallidis. 2007. Modelling the effect of high pressure on the inactivation kinetics of a pressure-resistant strain of *Pediococcus damnosus* in phosphate buffer and gilt-head seabream (*Sparus aurata*). *J. Appl. Microbiol.* 102:1499–1507.
29. Pao, S., and C. L. Davis. 1999. Enhancing microbiological safety of fresh orange juice by fruit immersion in hot water and chemical sanitizers. *J. Food Prot.* 62:756–760.
30. Patterson, M. F., M. Quinn, R. Simpson, and A. Gilmour. 1995. Sensitivity of vegetative pathogens to high hydrostatic pressure treatment in phosphate-buffered saline and foods. *J. Food Prot.* 58: 524–529.
31. Peleg, M., and M. B. Cole. 1998. Reinterpretation of microbial survival curves. *Crit. Rev. Food Sci. Nutr.* 38:353–380.
32. Polydera, A. C., N. G. Stoforos, and P. S. Taoukis. 2003. Comparative shelf life study and vitamin C loss kinetics in pasteurised and high pressure processed reconstituted orange juice. *J. Food Eng.* 60:21–29.
33. Polydera, A. C., N. G. Stoforos, and P. S. Taoukis. 2005. Quality degradation kinetics of pasteurised and high pressure processed fresh Navel orange juice: nutritional parameters and shelf life. *Innov. Food Sci. Emerg. Technol.* 6:1–9.
34. Sampedro, F., D. J. Geveke, X. Fan, and H. Q. Zhang. 2009. Effect of PEF, HHP and thermal treatment on PME inactivation and volatile compounds concentration of an orange juice–milk based beverage. *Innov. Food Sci. Emerg. Technol.* 10:463–469.
35. Setlow, R. B., and W. L. Carrier. 1966. Pyrimidine dimers in ultraviolet-irradiated DNA's. *J. Mol. Biol.* 17:237–254.
36. Truong, T., J. Boff, D. Min, and T. Shellhammer. 2002. Effects of carbon dioxide in high-pressure processing on pectinmethylesterase in single-strength orange juice. *J. Food Sci.* 67:3058–3062.
37. Uckoo, R. M., G. K. Jayaprakasha, J. A. Somerville, V. M. Balasubramaniam, M. Pinarte, and B. S. Patil. 2013. High pressure processing controls microbial growth and minimally alters the levels of health promoting compounds in grapefruit (*Citrus paradisi* Macfad) juice. *Innov. Food Sci. Emerg. Technol.* 18:7–14.
38. U.S. Food and Drug Administration. 2001. Hazard analysis and critical control point (HACCP); procedures for the safe and sanitary processing and importing of juice. *Fed. Regist.* 66:6138–6202.
39. van Boekel, M. A. J. S. 2002. On the use of the Weibull model to describe thermal inactivation of microbial vegetative cells. *Int. J. Food Microbiol.* 74:139–159.
40. Vega-Gálvez, A., C. Giovagnoli, M. Pérez-Won, J. E. Reyes, J. Vergara, M. Miranda, E. Uribe, and K. D. Scala. 2012. Application of high hydrostatic pressure to aloe vera (*Aloe barbadensis* Miller) gel: microbial inactivation and evaluation of quality parameters. *Innov. Food Sci. Emerg. Technol.* 13:57–63.
41. Walkling-Ribeiro, M., F. Noci, J. Riener, D. A. Cronin, J. G. Lyng, and D. J. Morgan. 2009. The impact of thermosonication and pulsed electric fields on *Staphylococcus aureus* inactivation and selected quality parameters in orange juice. *Food Bioprocess Technol.* 2:422–430.
42. Wang, L., J. Pan, H. Xie, Y. Yang, and C. Lin. 2010. Inactivation of *Staphylococcus aureus* and *Escherichia coli* by the synergistic action of high hydrostatic pressure and dissolved CO_2. *Int. J. Food Microbiol.* 144:118–125.
43. Wiley, R. 1994. Preservation methods for minimally processed refrigerated fruits and vegetables, p. 66–134. *In* R. C. Wiley (ed.), Minimally processed refrigerated fruits & vegetables. Springer, New York.
44. Zhao, L., S. Wang, F. Liu, P. Dong, W. Huang, L. Xiong, and X. Liao. 2013. Comparing the effects of high hydrostatic pressure and thermal pasteurization combined with nisin on the quality of cucumber juice drinks. *Innov. Food Sci. Emerg. Technol.* 17:27–36.
45. Zhao, L., Y. Wang, S. Wang, H. Li, W. Huang, and X. Liao. 2014. Inactivation of naturally occurring microbiota in cucumber juice by pressure treatment. *Int. J. Food Microbiol.* 174:12–18.
46. Zhao, T., M. P. Doyle, and R. E. Besser. 1993. Fate of enterohemorrhagic *Escherichia coli* O157:H7 in apple cider with and without preservatives *Appl. Environ. Microbiol.* 59:2526–2530.