Postharvest Biology and Technology 127 (2017) 21–26

Contents lists available at ScienceDirect

Postharvest Biology and Technology

journal homepage: www.elsevier.com/locate/postharvbio

Microwave-powered cold plasma treatment for improving microbiological safety of cherry tomato against *Salmonella*

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ARTICLE INFO

Article history:
Received 25 August 2016
Received in revised form 28 December 2016
Accepted 3 January 2017
Available online xxx

Keywords:
cherry tomato
cold plasma
nonthermal treatment
Salmonella

ABSTRACT

Microwave-powered cold plasma treatment (CPT) has been investigated as a nonthermal intervention technology for improving the microbiological safety of cherry tomatoes (*Solanum lycopersicum* var. *cerasiforme*) against *Salmonella* (approximately 7 log CFU/tomato). Cherry tomatoes were subjected to CPT using helium (He) or a He-oxygen (O_2) gas mixture for 2–10 min at 400–900 W of plasma generation power. A central composite method was applied to investigate the interactions between treatment conditions and the *Salmonella* reduction rate, weight loss, or temperature of the tomatoes. CPTs using He and a He-O_2 gas mixture at 827 W for 9 min resulted in the greatest reduction in *Salmonella* numbers (3.5 ± 0.1 and 3.5 ± 0.5 log CFU/tomato, respectively) and temperature increases of 3.0 ± 0.3 and 3.5 ± 0.4 °C, respectively. He-CPT at 900 W for 10 min, determined as the optimal conditions for *Salmonella* inactivation in this study, did not appreciably influence the surface morphology of cherry tomatoes. While the optimal He-CPT did not effectively inhibit the growth of *Salmonella* on the tomatoes at 25 °C, the treatment prevented the *Salmonella* growth during storage at 5 °C, without affecting the tomato respiration rate ($P < 0.05$). These results demonstrate the potential of CPT as a postharvest technology to improve the microbiological safety of cherry tomatoes against *Salmonella*, without affecting their biological properties.

1. Introduction

As grocery shoppers have begun to focus on convenience, healthiness, and freshness, the consumption of ready-to-eat (RTE) produce has increased continuously (Tian et al., 2012; Da Silva Felício et al., 2015). However, with the increased consumption of RTE produce, the incidence of food poisoning caused by foodborne pathogens, such as *Escherichia coli* O157:H7, *Salmonella* spp., and *Listeria monocytogenes*, has increased (Pagadala et al., 2015). Fresh vegetables are either directly contaminated by soil, irrigation water, animals, or insects, or cross-contaminated during harvesting, postharvest treatment, processing, or packaging (Koseki and Isobe, 2005). *Salmonella* on fruit and vegetables is reported to be resistant to decontamination by simple washing (Beuchat and Scouten, 2002; Aguiló-Aguayo et al., 2013). Cherry tomatoes were used in this study as they have recently been reported to be associated with salmonellosis outbreaks (Ziuzina et al., 2014).

Cold plasma (CP) treatment (CPT) is an alternative nonthermal processing technology for microbial decontamination of fresh fruit and vegetables (Niemira, 2012; Lee et al., 2015). The advantages of CPT over conventional techniques include its nonthermal and nontoxic nature and short treatment duration (Niemira, 2012; Ziuzina et al., 2012, 2014). The antimicrobial effectiveness of CPT is due to the chemical species, including free radicals, electrons, ionized molecules, and ultraviolet (UV) photons, generated during the process, which directly oxidize the lipids in bacterial cell walls and react with intracellular molecules such as proteins and nucleic acids (Gallagher et al., 2007; Ramazzina et al., 2015).

Recently, several studies have reported the use of CPT for microbial decontamination of fruit and vegetables: The numbers of *E. coli* in salad were reduced by 3.6 log CFU/cm^2 by treatment with an atmospheric CP jet using air as plasma-forming gas (Baier et al., 2013), and *Salmonella typhimurium* numbers in cabbage were reduced by 1.5 log CFU/g by microwave-powered CPT using nitrogen (N_2) at 900 W for 10 min (Lee et al., 2015). Dielectric barrier discharge CPT (air, 15 kV, 30 min) decreased the number of *L. monocytogenes* on red chicory leaves by 2.2 log CFU/cm^2 (Pasquali et al., 2016). Zhang et al. (2013) reported that a 10 min treatment with low-pressure radio frequency (RF) plasma (67 Pa, 13.56 MHz, 0.34 W/m^3) using O_2 reduced the number of *S. typhimurium* inoculated on tomatoes by 2.2 log CFU/cm^2. In a

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http://dx.doi.org/10.1016/j.postharvbio.2017.01.001
0925-5214/

similar experiment, Ziuzina et al. (2014) showed that 60 s of atmospheric CPT (air, 70 kV) reduced the number of *S. typhimurium* on tomatoes from 6.3 log CFU/sample to undetectable levels. However, a simultaneous assessment of the effects of CPT on microbial stability and the properties of produce during subsequent storage, e.g., respiration rate changes corresponding to the level of physiological stress generated in produce (Rico et al., 2007; Misra et al., 2014), is necessary for commercial produce application. In addition, further studies of decontamination of produce, preferably using a cocktail of strains, are required (Rød et al., 2012). Therefore, the objectives of this study were: 1) to optimize CPT conditions for maximum inactivation of *Salmonella* inoculated onto tomato, and 2) to investigate the effects of the optimal CPT on the growth of *Salmonella* and CO_2 generation during storage at 5 and 25 °C.

2. Materials and methods

2.1. Tomato preparation

Cherry tomatoes used in this study were purchased from Farm's Hill (Nonsan, Korea). The weight of a single cherry tomato was 10–12 g. Tomato stems were carefully removed before the tomatoes were pre-washed with distilled water, immersed in 200 mL of 70% (v/v) ethanol for 1 min, and then rinsed twice with 500 mL of sterile deionized water by light rubbing for 5 min. Tomatoes were placed on a sterile Petri dish (90 × 15 mm; SPL Life Science Co., Pocheon, Korea) (two in each dish). The tomatoes were dried in a laminar flow biohazard hood (SterilGARD, Baker Company Inc., Sanford, ME, USA) for 1 h at 25 ± 5 °C.

2.2. Microbial strains and inoculation

S. typhimurium (CCARM 8164), *S.* Enteritidis (CCARM 8040), and *S. enterica* subspecies *enterica* serovar Montevideo (CCARM 8052) were provided by the Culture Collection of Antibiotic-Resistant Microbes (Seoul Women's University, Seoul, Korea). Prior to experiments, frozen stock cultures of each strain were streaked on tryptic soy agar (TSA; Difco™, Becton and Dickinson, Detroit, MI, USA) and incubated at 37 °C for 24 h for a maximum concentration in early stationary phase (Wang et al., 2013). Subcultures were performed twice in tryptic soy broth (TSB; Difco™). *Salmonella* cultures were harvested and washed three times in 0.1% (w/v) sterile peptone water by centrifugation at 5,000 rpm (10 min). Equal volumes of each culture were collected to prepare the *Salmonella* cocktail.

The stem-off part of each cherry tomato was placed in a sterile Petri dish (SPL Life Science Co.) in a laminar flow biohazard hood, and the cocktail (250 μL; approximately 7.0 log CFU/mL) was spot-inoculated onto the top surface (~7.1 cm^2) and evenly spread using a sterile disposable spreader (SPL Life Science Co.) to cover about three quarters of the tomato surface. The tomatoes were then dried for 1 h at 25 ± 5 °C before CPT. The number of *Salmonella* on each tomato in the absence of CPT was 6.0 ± 0.4 log CFU/tomato.

2.3. CPT

A previously developed CPT system (Kim et al., 2014) that generates CP using microwave power was used in this study. The magnetron (Magnetron 2M246, LG electronics Inc., Seoul, Korea) in the microwave generator produces a 2.45-GHz wave discharge at 50–1000 W. The flow rate of plasma-forming gas is controlled by the gas mass flow rate controller (two channels, Model 3660, Kojima Instrument Inc., Osaka, Japan) at a maximum of 20 standard L/min. The pressure in the chamber ranges from 0.5 to 30 kPa. The dimension of the treatment chamber, made of stainless steel, is 43 cm (width) × 37 cm (height) × 40 cm (length). A cherry tomato was located 24 cm from the center bottom of the plasma treatment chamber (Kim et al., 2017). Helium (He) or a mixture of 99.8% He and 0.2% oxygen (O_2) (v/v), which formed a stable plasma (Kim et al., 2014), was used as the plasma-forming gas. He-O_2 gas mixture was used because O_2 enhances free radical generation (Kim et al., 2011). N_2 and an N_2-O_2 gas mixture were also tested, but resulted in an increase in the tomato surface temperature (>35 °C; data not shown). To optimize the CPT conditions, the level of power used to generate CP (CP generation power, X_1) (400, 473, 650, 827, or 900 W) and the treatment time (X_2; 2, 3, 6, 9, or 10 min) were varied. Untreated samples were kept in a vacuum (0.7 kPa) for the same amount of time as the CPTs. The treatment pressure and gas mass flow rate were 0.7 kPa and 1 L/min, respectively.

2.4. Analyses of Salmonella reduction, tomato weight change, tomato surface temperature change, and tomato surface morphology

The numbers of residual *Salmonella* were determined. Each cherry tomato was aseptically transferred to a sterile bag (30 mL, Nasco Whirl-Pak®, Fort Atkinson, WI, USA) to which 25 mL of sterile 0.1% peptone water was added. The bag was sealed and the tomato was gently rubbed manually using the inner surface of the bag for 3 min to transfer the bacteria to the liquid. The resulting microbial solutions were serially diluted in sterile 0.1% peptone water, and 100 μL of each of the diluted suspensions was spread on a xylose lysine deoxycholate agar (XLD; Difco™) plate.

Weight loss of cherry tomato was expressed as a percentage of the initial sample weight. An electronic balance (EK-200i, AND Inc., Tokyo, Japan) with an accuracy of ± 0.01 g was used for weight measurements. The temperature at the surface of the tomato was measured using an infrared thermometer (DT 44L, DIAS Infrared GmbH, Dresden, Germany).

The surface morphology of untreated tomatoes and those treated with He-CP using the optimal conditions (900 W, 10 min) was visualized by field emission-scanning electron microscopy (FE-SEM). Untreated tomato samples were not subjected to CPT, but were subjected to a low pressure of 0.7 kPa for 10 min. The edges of tomato skin slices (2 × 2 cm) were fixed on the cover of a sterile Petri dish (SPL Life Science Co.) parallel to the bottom of the dish, and 1 mL of 1% (w/w) osmium tetroxide (19192, Electron Microscopy Sciences, Hatfield, PA, USA) was evenly spotted on the bottom of the Petri dish. The cover of the Petri dish was replaced and plates were incubated overnight at 25 ± 2 °C. The surface morphology of tomato skin was visualized by FE-SEM (S-4700, Hitachi, Tokyo, Japan) at 10 kV and 1,000 × magnification.

2.5. Salmonella inhibition and CO_2 generation during storage after CPT

The effects of CPT using He (He-CPT) on *Salmonella* survival and CO_2 generation by tomatoes during subsequent storage were investigated. Optimal conditions determined in this study (900 W, 10 min) were used for CPT of tomatoes, while untreated tomatoes were incubated under the same pressure for an equal amount of time but not subjected to CPT. Tomatoes were subsequently stored at 5.0 ± 0.1 or 25.0 ± 0.2 °C to simulate the storage conditions commonly used by consumers (Nunes et al., 2009; Renard et al., 2013). The relative humidity in the bags (30 mL; Nasco Whirl-Pak®) containing cherry tomatoes at either temperature was 85 ± 5%. Treated and untreated *Salmonella*-contaminated tomatoes were stored separately for 28 days at 5 °C or 10 days at 25 °C.

The microbial analysis samples were prepared as described in section of 2.4. The number of *Salmonella* was analyzed by colony counting after plating on XLD (selective growth medium) and TSA (non-selective enriched growth medium) plates. Plates were