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Short communication

Inhibition of *Salmonella typhimurium* on radish sprouts using nitrogen-cold plasma

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ABSTRACT

This study investigated the effects of cold plasma treatment (CPT) on the inhibition of *Salmonella typhimurium* on radish sprouts and the quality attributes of the sprouts. Radish sprouts were treated with nitrogen (N_2)-cold plasma at 900 W and 667 Pa for 0, 2, 5, 10, and 20 min using a microwave-powered CPT system. The sensory attributes of the radish sprouts, appearance and odor, were evaluated before and after the treatment. The effects of N_2-CPT for 10 min on microbial growth and the quality attributes of the radish sprouts were evaluated during storage for 12 days at 4 and 10 °C. N_2-CPT at 900 W and 667 Pa for 20 min reduced the number of *S. typhimurium* by 2.6 ± 0.4 log CFU/g. The moisture content of the radish sprouts decreased with treatment time. The appearance and odor of the radish sprouts were not altered by CPT ($p > 0.05$) and this treatment did not affect the quality attributes of the sprouts in terms of color, ascorbic acid concentration, or antioxidant activity during storage at both 4 and 10 °C. These findings suggest that CPT has the potential to improve the microbiological safety of radish sprouts with reference to *S. typhimurium* during cold storage without significant detriment to its quality properties.

1. Introduction

Consumer demand for healthy, fresh, and convenience products has led to an increasing preference for minimally processed ready-to-eat (RTE) vegetables (Fajardo et al., 2015; Quested et al., 2010; São José et al., 2014). Vegetable sprouts, which are primarily consumed as RTE products, possess antioxidant properties and contain phytochemical substances such as glucosinolates (Clarke et al., 2008), phenolic compounds, and ascorbic acid (Takaya et al., 2003). However, foodborne pathogens can grow on the surface of fresh produce. Outbreaks associated with RTE fresh produce have been reported worldwide (Losio et al., 2015; Sant'Ana et al., 2014; Zhang et al., 2016). In the United States, the food poisoning accidents related to vegetable sprouts have caused 42 outbreaks, 1193 illnesses, 133 hospitalizations, and three deaths between 2000 and 2014 (CDC, 2016). In particular, *Salmonella* contamination resulted in 29 outbreaks, 1019 illnesses, 91 hospitalizations, and one death (CDC, 2016).

Organic acid treatments are relatively simple to decontaminate fruits and vegetables, but they can have negative effects on sensory features such as taste, fragrance, and color. Currently, the most efficient and widely used treatment for the microbial decontamination is the use of conventional sanitizers, such as hypochlorous acid (HClO). However, HClO treatment is reported to produce chlorophenol, chloroform, and carcinogens such as trihalomethane that could have adverse effects on consumer health (Chun et al., 2013; Gil et al., 2009). Despite considerable research effort aimed at developing antimicrobial intervention technologies for fresh produce, there is still a need for new, safe, affordable, and more effective interventions to remove or inhibit foodborne pathogens from at-risk produce (Niemira, 2012).

Cold plasma treatment (CPT) is a non-thermal preservation method that has been intensively investigated for the microbial decontamination of fresh fruits and vegetables (Fernández et al., 2013; Lee et al., 2015). Damage to the microbial cell membrane by radicals, ultraviolet (UV) photons, electrons, and charged particles in the plasma underlies the inactivation of microorganisms by cold plasma (Kim et al., 2014). Additionally, electrons, ions, and free radicals can cause surface lesions (etching) in membranes and damage them via direct bombardment, which can result in the death of these cells (Kvam et al., 2012; Yang et al., 2009).

Bermúdez-Aguirre et al. (2013) reported a ~1.6 log CFU/g reduction in the number of *Escherichia coli* on inoculated lettuce following treatment with dielectric barrier discharge (DBD) cold plasma at 12.83 kV for 10 min using argon as the plasma-forming gas. Similarly, the numbers of *Salmonella typhimurium* on RTE lettuce, strawberries, and potatoes decreased by 2.72 ± 0.31, 1.76 ± 0.67, and 0.94 ± 0.30 log CFU/sample, respectively, following treatment with DBD cold plasma formed

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with N_2 gas (Fernández et al., 2013). Under optimum power (400 W) and time (10 min) conditions, microwave-powered CPT inactivated *Listeria monocytogenes* on lettuce by 1.8 ± 0.2 log CFU/g (Lee et al., 2015). However, to date, little research has been conducted to assess the effects of CPT on the inactivation of microorganisms on vegetable sprouts. Thus, the objectives of this research were to study the effects of CPT on the inactivation of *S. typhimurium* on radish sprouts and the sensory attributes of the sprouts and to investigate its effects on the microbial and quality properties of radish sprouts during storage at 4 and 10 °C.

2. Materials and methods

2.1. Radish sprouts

Fresh-cut radish sprouts (Kaiware Daikon) were purchased from a local store one day prior to each experiment and stored at 4 °C until required. Radish sprouts were washed with running deionized water once and dried in a laminar flow biohazard hood (Type A/B3, NuAire, Inc., Plymouth, MN, USA) for 30 min and then used without further preparation to evaluate the effect of CPT on the inactivation of total mesophilic aerobes. To prepare the samples inoculated with *Salmonella*, the sprouts were washed by immersing them in a 8% (w/v) solution of sodium bicarbonate (Pure Baking Soda, Arm & Hammer™, Church & Dwight Co., Inc., Princeton, NJ, USA) for 5 min and then in sterile distilled water three times for 1 min each, with gentle swirling. The washed sprouts were air-dried on a clean bench for 1 h as a single layer.

2.2. Microbial strains and inoculum subculture

The microbial inhibition studies were performed using *S. typhimurium* DT 104, which was obtained from the Agricultural Biotechnology Culture Collection at Seoul National University (Seoul, Korea). Glycerol stocks of *S. typhimurium* cultures were preserved in cryovials and stored in a deep freezer (−80 °C) until required. The frozen stock was streaked on tryptic soy agar (TSA; Difco, Detroit, MI, USA) and incubated at 37 °C for 24 h. An isolated colony was transferred to tryptic soy broth (TSB, Difco) (10 mL). At two consecutive one-day intervals, further transfers were made in TSB using a sterile loop. *S. typhimurium* cells from overnight (18 h) culture were washed twice with 0.1% (w/v) sterile peptone water via centrifugation at 4000 ×*g* for 15 min at 22 °C and suspended in 0.1% peptone water (approximately 10^9 CFU/mL). The suspension was diluted with 0.1% peptone water to produce an inoculum broth at ~5 log CFU/mL.

2.3. S. typhimurium inoculation

The washed radish sprouts (50 ± 1 g) were dip-inoculated by immersion in inoculum broth (500 mL, 5 log CFU/mL) for 15 min. During immersion process, the broth containing the radish sprouts was stirred with a magnetic bar to ensure even inoculation. A pair of sterile forceps was used to disperse the sprouts manually in the broth. The inoculated samples were dried in the laminar flow biohazard hood for 1 h at 22 ± 1 °C and 30% relative humidity (RH). The final number of *S. typhimurium* in the inoculated samples after drying was ~4 log CFU/g radish sprouts.

2.4. CPT

The inoculated sprouts were spread onto Teflon plates (25 × 25 × 1 cm) as a single layer. The weights of sprouts on the plate for each treatment were 5 and 25 g for the microbial inactivation study and the storage study, respectively. The sprout samples were exposed to cold plasma in the treatment chamber (Fig. 1). The CPT system, previously described by Kim et al. (2014), contains a microwave generator, a cooling system, a treatment chamber, a gas mass flow controller, a vacuum pump, and a parameter controller in which a microwave generator (2.45 GHz) with a variable power of 50–1000 W creates the plasma excitation. The cold plasma was formed by the N_2 gas flowing at a rate of 1 L/min and the plasma generation power and treatment pressure were 900 W and 667 Pa, respectively. The CPT times used to prepare the cold plasma-treated sprouts in the microbial inactivation study were 0, 2, 5, 10, and 20 min and the treatment time of the samples used for the storage study was 10 min. Immediately before and after CPT, the temperature of the plasma-treated surface was measured using an infrared (IR) thermometer (DT 44 L; DIAS Infrared GmbH, Dresden, Germany).

2.5. Microbial analysis

Radish sprout samples were placed in a sterile bag (384 mL, Nasco Whirl-Pak®; Fort Atkinson, WI, USA), diluted tenfold with sterile 0.1% (w/v) peptone water, and then homogenized with a blender (Stomacher Lab Blender Model 400, Seward Medical; London, UK) for 3 min at normal speed (230 ± 5% rpm). The suspension was serially diluted in 0.1% (w/v) peptone water and the diluted samples were plated on xylose lysine deoxycholate (XLD; Difco) and plate count agar (PCA; Difco) for enumeration of *S. typhimurium* and total mesophilic aerobic bacteria. Prior to enumeration, the XLD and PCA plates were incubated for 24 h at 35 ± 2 °C. The recovery rate of inoculated *S. typhimurium* by stomaching without plasma treatment was approximately 25%.

2.6. Sensory evaluation

The appearance and odor of untreated radish sprout samples and the samples treated with cold plasma at 900 W for 10 min were evaluated on the day of treatment. The samples were not inoculated with *S. typhimurium*. Panelists were recruited from among students in the Department of Food Science and Technology at Seoul Women's University (Seoul, Korea). A total of 24 panelists aged 20–32 years participated in the evaluation. Each sprout sample was placed on a white plate (eight pieces on each plate) that was labeled with a random number. The attributes were evaluated without the radish sprouts being consumed using scales for appearance and odor that ranged from 1 (dislike extremely) to 9 (like extremely).

2.7. Weight loss, water activity, and color

Weight loss was calculated as the percentage loss of the initial sample mass using an electronic balance (EK-200i, AND Inc.; Tokyo, Japan) with a precision of 0.01 g. The moisture content and water activity (a_w) of each sprout sample were measured with a moisture content analyzer (i-Thermo 163L, Bel Engineering Inc.; Milano, Italy) and an a_w meter (Pawkit a_w meter, Decagon Devices Inc.; Pullman, WA, USA).

Hunter (*L*, *a*, and *b*) values for the radish sprout samples were determined using a colorimeter (Minolta Chroma Meter CR-400, Minolta Camera Co.; Osaka, Japan). Each measurement sample was prepared with 5 g of radish sprouts, which were placed on a standard white tile so that they completely covered the area of measurement to avoid obtaining color values reflected by the tile color. Both leaf and stalk were measured five times for each measurement sample. The colorimeter was calibrated using a white standard tile, Illuminate C, and a 2° standard observer.

2.8. Ascorbic acid concentration

Radish sprout samples (2 g) were cut and minced into small pieces using sterile scissors and homogenized in water (15 mL) at 5000 rpm for 30 s. The homogenized suspension was centrifuged at 10,000 ×*g* for 15 min and the supernatant was filtrated through a syringe filter (Dismic®-25CP, cellulose acetate, pore size: 0.45 μm, Advantec MFS; Inc., CA, USA) and then stored at −20 °C until required. The ascorbic acid concentration (mg/g) in the radish sprout samples was determined