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Short communication

Cold plasma treatment for the microbiological safety of cabbage, lettuce, and dried figs

Hanna Lee [a], Jung Eun Kim [a], Myong-Soo Chung [b], Sea C. Min [a,*]

[a] Department of Food Science and Technology, Seoul Women's University, Seoul, 139-774, Republic of Korea
[b] Department of Food Science and Engineering, Ewha Womans University, Seoul, 120-750, Republic of Korea

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Microwave-powered cold plasma treatment (CPT) was evaluated as a means to improve the microbiological safety of fresh vegetables and dried fruits. The CPT at 900 W, conducted for 10 min using nitrogen as a plasma-forming gas, inactivated *Salmonella* Typhimurium inoculated on cabbage and lettuce by approximately 1.5 log CFU/g. The CPT at 400–900 W and 667 Pa, conducted for 1–10 min using a helium–oxygen gas mixture, inactivated *Listeria monocytogenes* on cabbage by 0.3–2.1 log CFU/g in a time-dependent manner ($P < 0.05$). The Weibull model adequately described the inactivation of *L. monocytogenes* on cabbage by CPT. The CPT at the optimum conditions of treatment power (400 W) and time (10 min) inactivated *L. monocytogenes* on lettuce by 1.8 ± 0.2 log CFU/g. As the water activity of the dried figs increased from 0.70 to 0.93, the reductions in numbers of *Escherichia coli* O157:H7 and *L. monocytogenes* on figs increased from 0.5 to 1.3 log CFU/g and from 1.0 to 1.6 log CFU/g, respectively. The microbial inactivation by CPT increased synergistically when the pH of the figs was reduced from 6 to 4. CTPs have potential application to increase the microbiological safety of vegetables and dried fruits.

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1. Introduction

Fresh produce is increasingly recognized as an important source of foodborne disease outbreaks in many countries (Gajraj et al., 2012). In the United States, the proportion of outbreaks associated with fresh produce, from all reported foodborne outbreaks with an identified food source, has increased from 0.7% in the 1970s to 6% in the 1990s (Sivapalasingam et al., 2004), to 13% in the 2000s (Doyle and Erickson, 2008), and to 33% in 2011 (CDC, 2012).

Dried figs, a popular dried fruit in the United States, Turkey, Greece, and Spain, are also subjected to contamination by foodborne pathogens, which occurs during harvesting, drying, storage, transportation and handling (Akbas and Ozdemir, 2008). Increase in the relative humidity of the atmosphere in contact with dried fruits during storage and distribution will increase in their water activity (a_w) affecting microbiological quality and safety (Moraga et al., 2011). Figs are reportedly contaminated with *Escherichia coli* as well as *Bacillus cereus*, and damp storage conditions can cause the bacteria to grow to a level of 10^7–10^8 CFU/g dried figs (Akbas and Ozdemir, 2008).

Cold plasma treatment (CPT) can inactivate microorganisms contaminating food products without a marked temperature increase (Fernandez et al., 2011). The cold plasma contains energetic reactive species, such as ultraviolet (UV) photons, electrons, positive and negative ions, free radicals, and excited or non-excited molecules and atoms. Surface erosion and oxidation of microbial cell membranes by reactive species and DNA modifications by UV are the proposed mechanisms for the inactivation of microorganisms by cold plasma (Laroussi and Leipold, 2004; Gallagher et al., 2007). Recently Baier et al. (2013) reported a ~4 log CFU/cm^2 reduction of *E. coli* on corn salad leaves by a radio frequency (RF, 27.12 MHz)-driven atmospheric pressure plasma jet at 20 W for 60 s using argon as a plasma-forming gas. Zhang et al. (2013) presented a microbial reduction (~3 log cycles) of *Salmonella* Typhimurium LT2 attached to spinach by cold plasma generated with oxygen in vacuum at 0.34 W/cm^3 of RF (13.56 MHz) and concluded that the CPT was more effective than washing with 3% hydrogen peroxide on decontaminating *S.* Typhimurium LT2 on spinach.

Extensive studies of the effects of the a_w of foods on the inactivation of microorganisms by various food preservation methods have been conducted (Gaillard et al., 1998; Aronsson and Rönner, 2001; Staack et al., 2008). Low a_w environments were reported to offer considerable protection against microorganisms when

* Corresponding author. Tel.: +82 2 970 5635; fax: +82 2 970 5977.
E-mail address: smin@swu.ac.kr (S.C. Min).

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using heat (Gaillard et al., 1998; Carlson et al., 2005), pressure (Palou et al., 1997; Setikaite et al., 2009), and pulsed electric fields (Min et al., 2002). The antimicrobial effects of those methods are also affected by the pH of foods. Changes in pH induce changes in the protonation of biologically active molecules on the surface of the microorganisms with a subsequent change in the sensitivity of the microorganisms to environmental or processing factors (Vega-Mercado et al., 1996). Nonetheless, the effects of a_w and pH of food on the inactivation of microorganisms in foods by CPT have not been reported. Thus, the objectives of this research were to (1) study the effects of CPTs on the inactivation of *S.* Typhimurium, *Listeria monocytogenes*, and *E. coli* O157:H7 inoculated on cabbage, lettuce, and dried figs, evaluating kinetic models for the inactivation of *L. monocytogenes* inoculated on cabbage and (2) investigate the effects of a_w and pH on the inactivation of *E. coli* O157:H7 and *L. monocytogenes* by CPT.

2. Materials and methods

2.1. Bacterial strains and inoculum preparation

S. Typhimurium DT 104, *L. monocytogenes* KCTC 3569, and *E. coli* O157:H7 ATCC 35150 were obtained from the Agricultural Biotechnology Culture Collection at Seoul National University (Seoul, Korea). The strains were stored at −80 °C in a deep freezer (KS70GR50, CryoCare™, Key Science Products Inc., TX, USA) and thawed on ice before use. Tryptic soy agar (TSA) (BD Difco™, Spark, MD, USA) and tryptic soy broth (TSB) (BD Difco™) were used as growth media for all strains. Prior to each experiment, frozen stock cultures of *S.* Typhimurium, *L. monocytogenes*, and *E. coli* O157:H7 were streaked on TSA. Cultures were incubated at 37 °C for 24 h and an isolated colony of each pathogen was transferred to TSB (10 mL). At two consecutive 24-h intervals, further transfers were made in TSB using a sterile loop. Cells from each overnight (18 h) culture were collected by centrifugation (4000 × *g*, 15 min, 22 °C) (GyroSpin, Gyrozen Co. Ltd., Seoul, Korea) and suspended in 0.1% peptone water. The suspension (approximately 10^9 CFU/mL) was diluted in 0.1% peptone water to produce the desired concentration of inoculum of each pathogen.

2.2. CPT system

The SWU-2 CPT system, illustrated in Fig. 1, consisted of microwave-generating parts, a cooling system, a treatment chamber, a gas mass flow rate controller, a vacuum pump, and a parameter controller (Fig. 1). The magnetron (Magnetron 2M246, LG electronics Inc., Seoul, Korea) in the microwave-generating parts produces a 2.45-GHz wave discharge operated at the 50–1000 W power level. The treatment chamber is made of stainless steel and measured 43 cm (width) × 37 cm (height) × 40 cm (length) with fused silica (quartz) observation windows. Cooling water flows at 0.8 m^3/min. The plasma-forming gas, nitrogen (N_2) or the mixture of helium (He) and oxygen (O_2) (He:O_2 = 99.8:0.2), flows at a maximum of 20 standard liter/min (slm), which is controlled by a gas mass flow rate controller (two channels, Model 3660, Kojima Instrument Inc., Osaka, Japan). The pressure in the chamber ranges from 500 to 30,000 Pa, which is adjusted by a vacuum valve (Model two-way electric ball valve, DongjooAP, Incheon, Korea).

2.3. Effect of CPT on the inactivation of S. Typhimurium and L. monocytogenes on cabbage and lettuce

Cabbage (*Brassica oleracea* var. *capitata* L.) and lettuce (*Lactuca sativa* L.) were purchased from a local store. Cabbage and lettuce

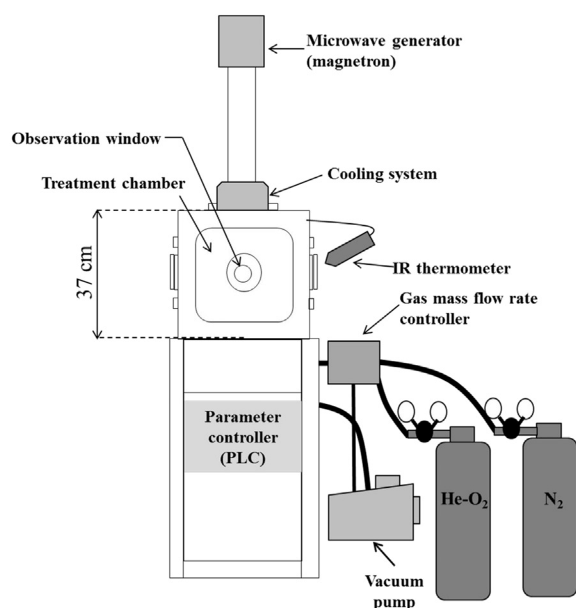

Fig. 1. Schematic diagram of the cold plasma treatment system.

were washed in an 8% (w/w) sodium hydrogen carbonate solution to decontaminate indigenous microorganisms. The indigenous aerobic population of washed cabbage and lettuce determined by plating on plate count agar (BD Difco™) was ~3 log CFU/g. The vegetables were cut into disks (6.0-cm diameter) using a cheese borer sterilized with ethanol. The weight of cabbage and lettuce disks ranged from 2.5 to 3.0 g. A 1.0-mL suspension of *S.* Typhimurium or *L. monocytogenes*, ~7 log CFU/mL, was inoculated on the disk samples using a sterile glass sprayer (BT1270S-100, Joylab Co., Seoul, Korea) and then dried for 1 h at 22 ± 2 °C and a relative humidity (RH) of 30% in a laminar flow biohazard hood (SterilGARD, Baker Company, Inc. Sanford, ME, USA).

The CPT conditions used for inactivation of *S.* Typhimurium and *L. monocytogenes* on cabbage and lettuce are presented in Table 1. The conditions were determined based on the results of preliminary studies. The CPTs using N_2, a N_2–O_2 mixture (N_2:O_2 = 99.3:0.7), He, and the He–O_2 mixture inactivated *S.* Typhimurium inoculated on lettuce by 1.6 ± 0.3, 1.3 ± 0.2, 0.4 ± 0.2, and 0.2 ± 0.2 log CFU/g, respectively. The mixture of He and O_2 exhibited a higher reduction of *L. monocytogenes* in cabbage (2.1 log CFU/g in max) than N_2 (0.5 log CFU/g). Thus, the N_2 and the H_2–O_2 mixture were selected as the plasma-forming gas for inactivating *S.* Typhimurium and *L. monocytogenes*, respectively. The pressure in the chamber and the gas flow rate were 667 Pa and 1 L/min, respectively, for all cabbage and lettuce treatments.

The effect of the treatment power and time on the inactivation of *L. monocytogenes* on cabbage was investigated by setting them as treatment variables. Pearson's correlation coefficients among the variables were determined using the SAS software (ver. 9.2, SAS Institute Inc., Raleigh, NC, USA). The experiment for studying the effect was designed using a response surface method (RSM) by Minitab (ver. 15, Minitab, Inc., State College, PA, USA). A two-variable, five-level central composite RSM design was employed (Nath et al., 2007), in which the explanatory variables were treatment power (X_1, 400, 474, 650, 826, 900 W) and treatment time (X_2, 1.0, 2.3, 5.5, 8.8, 10 min). The response variable was reduction (log reduction) in the level of *L. monocytogenes*. The effect of individual linear, quadratic, and interaction terms was determined using SAS.

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