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SHORT COMMUNICATION

Effect of atmospheric pressure plasma jet on the foodborne pathogens attached to commercial food containers

Hyun-Joo Kim[1] · Dinesh D. Jayasena[2,3] · Hae In Yong[4] · Amali U. Alahakoon[2] · Sanghoo Park[5] · Jooyoung Park[5] · Wonho Choe[5] · Cheorun Jo[4]

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Abstract Bacterial biofilms are associated with numerous infections and problems in the health care and food industries. The aim of this study was to evaluate the bactericidal effect of an atmospheric pressure plasma (APP) jet on *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* Typhimurium biofilm formation on collagen casing (CC), polypropylene (PP) and polyethylene terephthalate (PET), which are widely used food container materials. The samples were treated separately with the APP jet at a 50-W input power for 5 and 10 min, and nitrogen (6 l per minute) gas combined with oxygen (10 standard cubic centimeters per minute) was used to produce the APP. The APP jet reduced the number of bacterial cells in a time-dependent manner. All pathogens attached to CC, PP, and PET were reduced by 3–4 log CFU/cm^2 by the 10-min APP treatment. The developed APP jet was effectively reduced biofilms on CC, PP, and PET.

Keywords Biofilm · Food container · Atmospheric pressure plasma

Research Highlights
- Bactericidal effect of an atmospheric pressure plasma (APP) jet was evaluated.
- Biofilm was formed with 3 pathogens on 3 food containers.
- All pathogens on food containers were reduced 3–4 log CFU/cm^2 by 10-min treatment.
- APP jet was effectively reduced the biofilm on food containers.

✉ Cheorun Jo
cheorun@snu.ac.kr

[1] Crop Post-harvest Technology Division, National Institute of Crop Science, RDA, Suwon 16613, Republic of Korea

[2] Department of Animal Science and Biotechnology, Chungnam National University, Daejeon 34134, Republic of Korea

[3] Department of Animal Science, Uva Wellassa University, Badulla 90000, Sri Lanka

[4] Department of Agricultural Biotechnology, Center for Food and Bioconvergence, and Research Institute of Agriculture and Life Science, Seoul National University, Seoul 08826, Republic of Korea

[5] Department of Physics, Korea Advanced Institute of Science and Technology, Daejeon 34141, South Korea

Introduction

Microbial attachment to surfaces and the development of biofilms occur in many environments. Biofilms can be broadly defined as bacterial cells that attach to a surface and are frequently embedded in a polymer matrix of microbial origin (Denes et al. 2001). Bacterial biofilm formation is a serious concern in food processing facilities because it can cause cross-contamination, which may lead to food spoilage or the transmission of foodborne disease, which has health and economic consequences (Wang et al. 2003). Once microorganisms grow into a firm biofilm, cleaning and disinfection become much more difficult (Wirtanen et al. 2001). The cells associated with biofilms have advantages in growth and survival over planktonic cells due to the formation of an exopolysaccharide (EPS) matrix that surrounds biofilms, protecting them from sanitizers and supplying nutrients (Byun et al. 2007). Therefore, the rupture and removal of the EPS matrix is crucial.

Various studies have been undertaken to test methods for controlling microbial attachment and biofilm formation. Most methods rely on the use of various chemicals including chlorine (Schlisselberg and Yaron 2013). Chlorine compounds such as sodium hypochlorite can depolymerize EPS and are therefore used as disinfectants. Their bactericidal effects are based on the penetration power of the chemicals and oxidative action on essential cell enzymes (Lomander et al. 2004). However, these methods are limited because they produce

only a low log reduction in pathogen counts and leave residual toxic chemical reagents (Wang et al. 2003). Studying *Listeria innocua* in suspension, Byun et al. (2007) reported a reduction from 7.1 to 5.6 log colony-forming units (CFU)/mL after treatment with 100 ppm sodium hypochlorite, and no bacteria were detected after treatment 200 ppm sodium hypochlorite.

Atmospheric pressure plasma (APP) has been investigated as a novel non-thermal microbial inactivation technique in the field of food processing. Plasma is generated by introducing a gas into electromagnetic fields. Gas ions are generated by liberating electrons from the gas molecules when the field is strong enough (Heuer et al. 2015). The highly reactive free radicals and H_2O_2 produced during APP generation play a major role in bacteria inactivation (Yong et al. 2015). Reactive oxygen species affect bacterial membrane lipids through the formation of unsaturated fatty acid peroxides. The oxidation of amino acids and nucleic acids may also cause changes that result in microbial death or injury (Yun et al. 2010).

Various APP devices with distinct characteristics have recently been developed. The jet- type APP is used most frequently because it offers stable discharge, low gas temperature, and high concentrations of reactive species. Several researchers have studied the effect of APP on pork and bacon and demonstrated the potential applications of APP on food processing (Moon et al. 2009; Kim et al. 2013). However, few studies have examined the effects of APP on microbial biofilms-especially those of foodborne pathogens-attached to food container materials. In general, mechanical procedures are used to remove biofilms on these materials because antimicrobial agents have less penetration power for biofilm inactivation (Ramage et al. 2001; Hoiby et al. 2011).

The objective of the present study was to evaluate the bactericidal effect of an APP jet on *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* Typhimurium biofilms attached to food packaging material surfaces including collagen casing (CC), polypropylene (PP), and polyethylene terephthalate (PET).

Materials and methods

Materials and sterilization

CC, PP, and PET were purchased from a local store and cut into pieces (1 × 1 cm). The pieces were then washed twice with 70 % ethanol and dried at room temperature. For the inoculation test, the material samples were sterilized with electron beam irradiation (35 kGy at 10 MeV) in a linear electron-beam radio frequency accelerator (EB tech, Daejeon, Korea).

Test pathogens and culture conditions

E. coli O157:H7 (ATCC 43894), *L. monocytogenes* (KCTC 3569), and *S.* Typhimurium (KCTC 1925) were cultivated in tryptic soy broth, tryptic soy broth containing 0.6 % yeast extract, and nutrient broth, respectively (Difco, Detroit, MI, USA) at 37 °C for 24 h. The activated cell cultures were then centrifuged (2795 × *g* for 10 min) at 4 °C in a refrigerated centrifuge (VS-5500, Vision Scientific Co., Seoul, Korea), and the resultant pellets were washed twice with sterile saline solution (0.85 %). The pellets were then suspended in sterile saline solution to a final cell concentration of approximately 10^8–10^9 CFU/mL.

Inoculation of test pathogens and biofilm formation

The sterilized CC, PP, and PET samples were separately immersed in containers with a suspension of each test culture (25 mL) and incubated at 37 °C for 24 h. Then, the samples were air-dried in a clean bench for 30 min.

APP jet treatment

The APP jet device used in this study was devised according to the process for generating arc plasma. The anode electrode was cylindrical with a sharpened tip, and the diameter of the emission hole was 1.5 mm (Fig. 1). The electrode was covered with a cathode nozzle combined with a cooling system. The plasma was generated at 0.5-kW peak power, and nitrogen [6 l

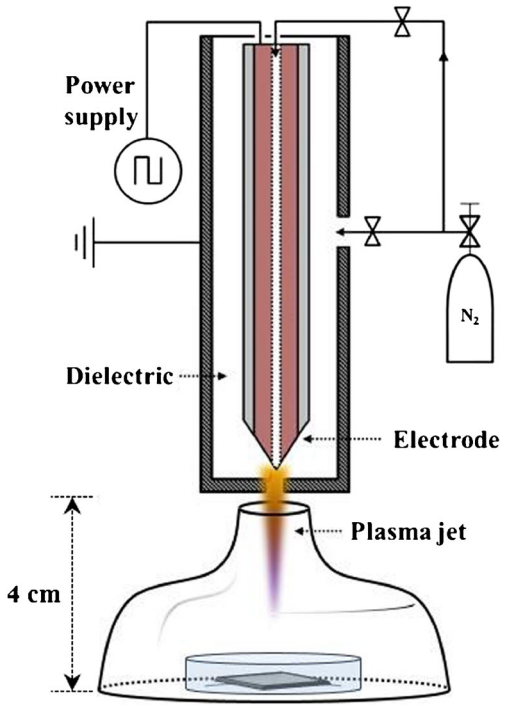

Fig. 1 Schematic diagram of the experimental setup for generation of atmospheric pressure plasma

per minute (lpm)] and oxygen [10 standard cubic centimeter per minutes (sccm)] were used as carrier gases (Yong et al. 2014).

The samples inoculated with the test pathogens were covered with a glass cone (Fig. 1) and treated with APP for 0, 5, or 10 min. After APP treatment, the samples were immediately stored at 25 °C for 0, 3, and 7 days.

Microbial analysis

The APP-treated samples were transferred to Petri dishes and rinsed with sterile saline solution (0.85 %) (Byun et al. 2007). After drying for 10 min, the samples were sonicated (Branson Ultrasonic Co., Danbury, CT, USA) in saline solution for 5 min and vortexed for 1 min to detach the bacterial cells. After serial dilutions were prepared, 100-μL aliquots from each dilution were spread on plates containing the appropriate medium according to a standard spread-plating method. Each experiment was performed in triplicate. Tryptic soy agar, tryptic soy agar containing 0.6 % yeast extract, and nutrient agar (Difco) were used to enumerate *E. coli* O157:H7, *L. monocytogenes*, and *S.* Typhimurium, respectively. The plates were incubated at 37 °C for 48 h, and the microbial counts were expressed as log CFU/cm^2.

Statistical analysis

All experimental procedures were performed in triplicate with three observations. The data were analyzed with one-way analysis of variance using the general linear model, and significant differences among mean values were identified by using the Student-Newman-Keul's multiple range test in SAS Release 9.2 (SAS Institute Inc., Cary, NC, USA) at a confidence level of $P < 0.05$.

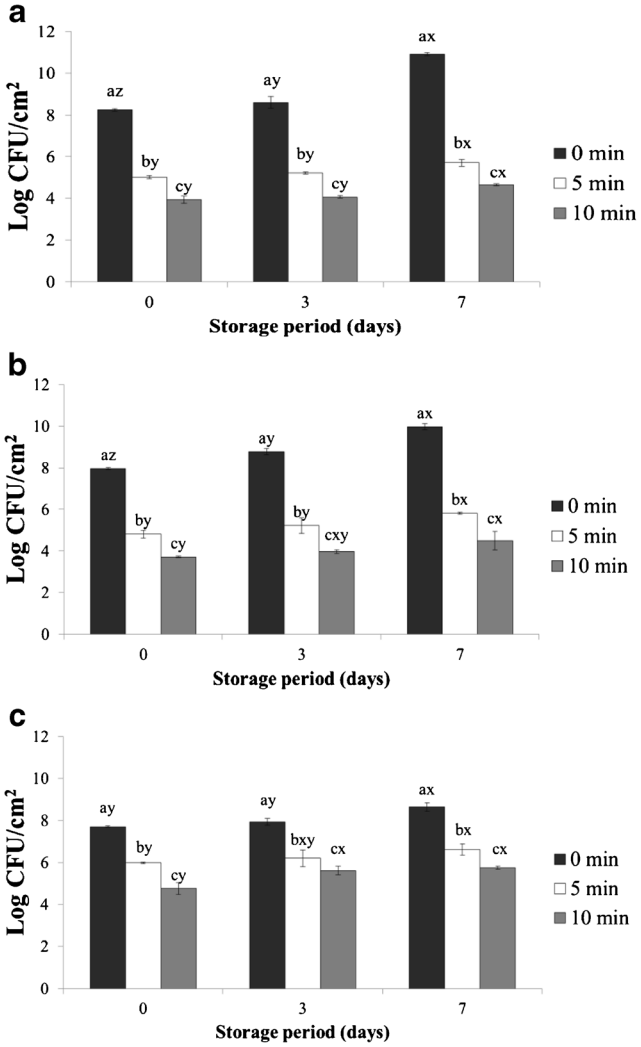

Fig. 2 *Escherichia coli* O157:H7 counts (log CFU/cm^2) in the biofilms treated with atmospheric pressure plasma jet for 0, 5, and 10 min. **a** Collagen casing, **b** Polyethylene terephthalate, **c** Polypropylene. ($^{a\text{-}c}$Values with different letters within the same storage day differ significantly ($P < 0.05$); $^{x\text{-}z}$Values with different letters within the same plasma treatment time differ significantly ($P < 0.05$))

Results and discussion

Figure 2 shows the reductions in bacterial counts of *E. coli* O157:H7 biofilm after APP jet treatment. The overnight-inoculated counts were 8.25, 7.97, and 7.72 log CFU/cm^2 in CC, PET, and PP, respectively. APP jet treatment significantly reduced the number of *E. coli* O157:H7 cells in a time-dependent manner. The number of *E. coli* O157:H7 cells increased with the increase in storage period ($P < 0.05$). Counts of *L. monocytogenes* attached to CC, PET, and PP were significantly reduced by the APP jet treatment (Fig. 3). When untreated CC, PET, and PP were stored for 7 days, the *L. monocytogenes* counts increased significantly by 2.13, 1.95, and 1.69 log CFU/cm^2, respectively. However, *L. monocytogenes* counts increased by only 0.74, 0.95, and 0.52 log CFU/cm^2 on CC, PET, and PP treated with APP for 10 min, respectively. The reduction pattern of *S.* Typhimurium counts after plasma treatment showed a trend similar to those of *E. coli* O157:H7 and *L. monocytogenes* (Fig. 4). The 10-min APP treatment reduced the number of *S.* Typhimurium on CC, PET and PP by 3.87, 4.48, and 4.32 log CFU/cm^2, respectively.

Several studies have demonstrated the effectiveness of plasma treatment for microbial inactivation in food packaging materials. Muranyi et al. (2010) reported the use of cold plasma treatments for the sterilization of PET foils and polystyrene with attached *Bacillus atrophaeus* spores and vegetative cells. Lee et al. (2011) reported that plasma generated with mixed gas decreased *L. monocytogenes* counts on casing by 0.50 ± 0.30 log CFU/cm^2.

Only a few studies thus far have examined the use of plasma for the disinfection or inactivation of biofilms on food

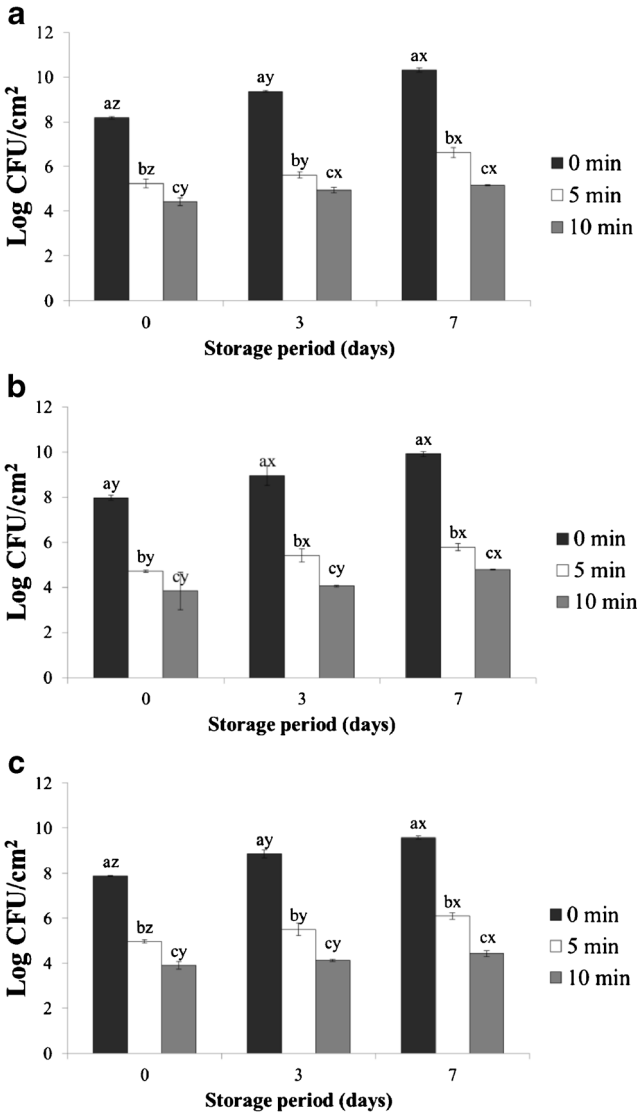

Fig. 3 *Listeria monocytogenes* counts (log CFU/cm^2) in the biofilms treated with atmospheric pressure plasma jet for 0, 5, and 10 min. **a** Collagen casing, **b** Polyethylene terephthalate, **c** Polypropylene. ($^{a\text{-}c}$Values with different letters within the same storage day differ significantly ($P < 0.05$); $^{x\text{-}z}$Values with different letters within the same plasma treatment time differ significantly ($P < 0.05$))

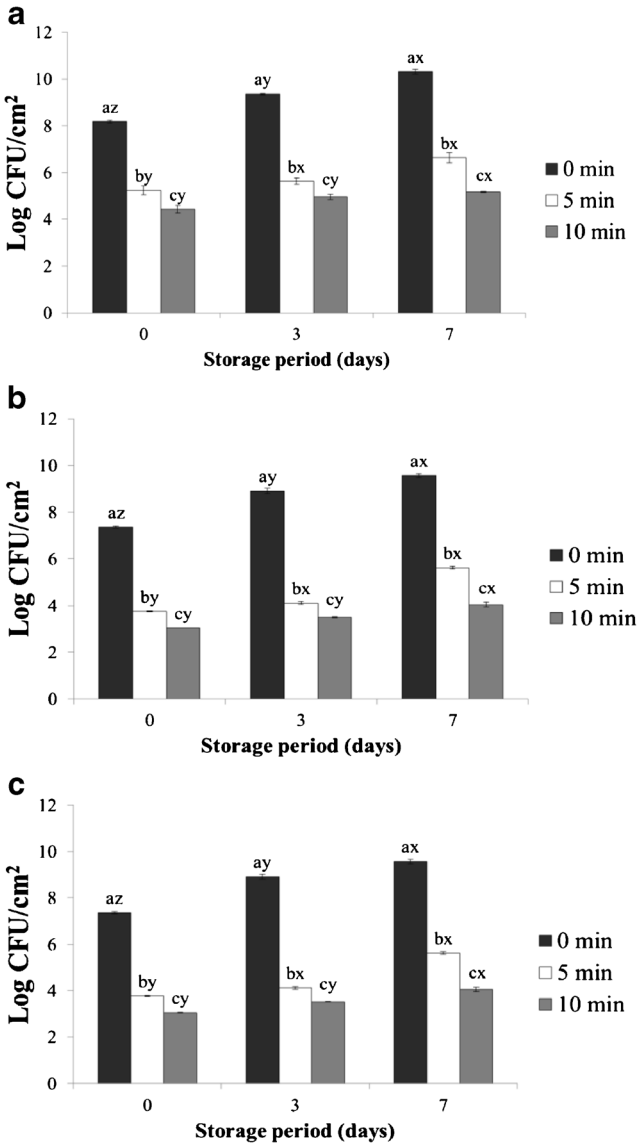

Fig. 4 *Salmonella* Typhimurium counts (log CFU/cm^2) in the biofilms treated with atmospheric pressure plasma jet for 0, 5, and 10 min. **a** Collagen casing, **b** Polyethylene terephthalate, **c** Polypropylene. ($^{a\text{-}c}$Values with different letters within the same storage day differ significantly ($P < 0.05$); $^{x\text{-}z}$Values with different letters within the same plasma treatment time differ significantly ($P < 0.05$)

container materials. Vleugels et al. (2005) successfully reduced biofilm-forming *Pantoea agglomerans* (commonly associated with plant tissues) grown on synthetic membranes by two orders of log reduction with a 10-min glow discharge plasma treatment. Furthermore, they reported an insignificant color change in bell pepper samples after exposure to plasma. Factors that influence microbial biofilm formation on a surface include the growth phase of the cells, the type and properties of the inert material, the presence of organic materials, environmental pH, and temperature (Stone and Zottola 1985; Byun et al. 2007). Although the mechanisms underlying these effects are not always known, biofilm formation can be influenced in some cases by altering the bacterial cell surface (Van Houdt and Michiels 2010). Additionally, the authors suggested that the differences in the efficacy of APP among the cells in biofilms were attributable to the limited penetration of each plasma type into the biofilms.

During the plasma treatment process, an oxygen functional group forms at the surface of treated materials through interactions between the active species from the plasma and the surface atoms of the materials (Guruvenket et al. 2004). Fricke et al. (2012) applied APP jet treatment to inactivate *Candida albicans* biofilms on polystyrene wafers and observed almost complete biofilm elimination when oxygen was added to argon as a plasma discharge gas. The combination of argon and

oxygen has higher chemical activity due to the formation of reactive atomic and molecular radicals that interact with organic materials. In plasma-solid interactions, plasma processing may chemically and physically modify the material surface (Pykonen et al. 2008). In particular, the groups responsible for surface hydrophilicity-such as peroxide, carboxylic acid, ketone/aldehyde, and ester groups-can be generated and activated on the surfaces by plasma (Lai et al. 2006).

Plasma treatments significantly increase the hydrophilicity and adhesion capability of film surfaces due to the formation of polar substances (Theapsak et al. 2012). In our study, the hydrophobicity of food containers and materials might have been activated by the APP jet. This activation may have occurred in the thin surface layer as a consequence of processes such as oxidation, degradation, cross-linking, and structural change. The efficiency of these processes depends on the gas pressure, temperature, solid surface characteristics, monomers used for plasma polymerization, power, and time of plasma action (Kaminska et al. 2002; Lee et al. 2012).

Conclusion

APP jet treatment significantly reduced the pathogenic biofilms attached to CC, PP, and PET. Thus, APP jet treatment is as an alternative non-thermal technology for the reduction of pathogenic biofilms. However, we observed differences in the reduction of the microbial load attached to CC, PP, and PET, indicating that the resistance of the pathogenic biofilms on the food packaging materials is different.

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