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Evaluation of the Treatment of Both Sides of Raw Chicken Breasts with an Atmospheric Pressure Plasma Jet for the Inactivation of *Escherichia coli*

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Abstract

Atmospheric pressure plasma (APP) is an emerging nonthermal microbial inactivation technique. In this study, agar and raw chicken breast were inoculated with *Escherichia coli* and treated with an APP jet based on cold arc plasma. The aim of this study was to investigate the optimum conditions for the plasma treatment of an APP jet in order to maximize the efficiency of *E. coli* inactivation. The combination of N_2+O_2 (10 standard cubic centimeters per minute) and a longer treatment time (10 min) resulted in the highest inactivation of *E. coli* on agar plates with an optimum treatment distance of 20 mm. The samples in dry and wet conditions showed similar reductions in *E. coli* count when one side of the samples was treated at a given treatment time. Treating both sides—2.5 min on each side—resulted in a higher growth inhibition of *E. coli* than treatment of a single side only for 5 min. However, there was no significant difference between one-side treated samples (10 min) and both-sides treated samples (5+5 min). When the concentration of *E. coli* in the chicken breast sample was 10^4 colony-forming units (CFU)/g, the reduction rate of the *E. coli* was the highest, followed by 10^5, 10^6, and 10^7 CFU/g; however, no difference was found between 10^3 and 10^4 CFU/g. In conclusion, various treatment conditions may affect the inactivation efficiency of *E. coli.* In the present study, the optimum condition was determined as the treatment distance of 20 mm and longer treatment time (10 min) with the addition of oxygen to the nitrogen gas flow. Furthermore, the cell concentration of sample was an important parameter for the efficacy of the inactivation process.

Introduction

Consumers demand safer food and do not wish to purchase food with potential health hazards, such as foodborne pathogens. Raw meat products that have not undergone any further preparation or cooking are at a higher risk of contamination, and the number of records describing outbreaks of foodborne illness associated with raw products has increased (Bolder, 1997; FDA, 2008). Consumers usually perceive poultry as a healthy, cheaper, and ''easy-to-prepare'' food, although its consumption is often related to foodborne disease and occasionally to severe outbreaks, mainly caused by *Campylobacter* spp., *Salmonella* spp., or even *Listeria monocytogenes* and *Escherichia coli* (van den Bogaard *et al.*, 2001; EFSA, 2012).

In several countries, the application of physical or chemical decontamination treatments to poultry carcasses is permitted after slaughtering and prior to or during the chilling stage, thus combining their separate antimicrobial effects. Chemical decontamination techniques such as organic acids, phosphates, or chlorine-based solution, among others, have proved effective in reducing the microbial load of chicken meat stored at low temperatures (Loretz *et al.,* 2010). The problem unfortunately is not only restricted with chlorine and surviving bacteria, but also residues and antimicrobial resistance of the bacteria. Thus, current European regulations on meat hygiene do not allow any treatment other than portable water or steam (Alonso-Hernando *et al.*, 2013). Therefore, successful elimination of bacteria from raw poultry products is challenging.

Atmospheric pressure plasma (APP) is an emerging nonthermal microbial inactivation technique. APP is an ionized gas, which consists of different antimicrobial substances, including charged particles, ultraviolet (UV) photons, and

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reactive species (Moisan *et al*., 2002; Deng *et al*., 2006; Misra *et al*., 2011). There are different types of APP with their own characteristics (Dirks *et al*., 2012). APP jet is used because of its stable discharge, low gas temperature, and high concentrations of reactive species (Schutze *et al*., 1998; Walsh *et al*., 2006). Highly reactive species can overcome natural defense mechanisms, resulting in damage to DNA, proteins, lipids, and membranes (Kim *et al.*, 2013). The ability of APP to reduce foodborne pathogens on the surface of products has been examined, and it has been revealed that APP has bactericidal, virucidal, and fungicidal properties (Deng *et al*., 2006; Lee *et al*., 2006; Moreau *et al*., 2008; Fernández *et al*., 2012). Therefore, the generation and utilization of plasma may be competitive due to its simplicity and cost-effectiveness compared to other microbial inactivation methods (Kim *et al.,* 2013). Lee *et al*. (2011, 2012) reported that *L. monocytogenes* inoculated onto slices of cooked chicken breast, ham, and cooked egg white and yolk was efficiently reduced by an APP jet. Similar results were observed in chicken meat and skin inoculated with *L. innocua* and exposed to an APP jet (Noriega *et al*., 2011).

Most of these previous studies were, however, limited in their ability to confirm the inactivation of microorganisms by APP. Experimental conditions, including plasma physics (gas composition, flow rate, input power, and type of discharge) and molecular microbiology (gram negative or positive, bacteria or fungi, and cell concentration of microorganism), may influence the inactivation efficiency of APP treatment (Deng *et al*., 2006; Fernández and Thompson, 2011; Fernández *et al*., 2013). A recent study suggests that the bacterial growth phase and growth temperature play a minor role in the inactivation of *Salmonella* Typhimurium by APP jet with nitrogen (Fernández *et al*., 2012). It is thus important to find the best plasma conditions for its optimum inactivation efficiency.

The main objective of the present study was to investigate the optimum conditions to maximize *E. coli* inactivation efficiency, including distance of the sample from the plasma jet, gas composition, humidity of the sample, and the microbial cell concentration. The inactivation effect of plasma treatment on both sides of the samples was also studied for its use in practical situations.

Materials and Methods

Sample preparation and sterilization

Raw chicken breasts with the skins still on were purchased from a local market in Daejeon, Korea. Prior to the inoculation, the samples were sterilized using electron-beam irradiation (35 kGy) with a 2.5 MeV linear electron beam radiofrequency accelerator (EB-Tech, Daejeon, Korea). To confirm the target dose, alanine dosimeters attached to the top and bottom surfaces of the sample pack were read using a 104 Electron Paramagnetic Resonance unit (EMS-104; Bruker Instruments Inc., Billerica, MA). The calculated maximum/minimum dose ratio was less than 1.004 for all the samples. Tryptic soy agar plates (50 × 10 mm; Difco, Becton Dickinson, Sparks, MD) were also prepared.

Microorganisms and inoculation

E. coli (KCTC 1682, serotype, serogroup O6, from clinical source) was obtained from the Korean Collection for Type Culture (KCTC, Daejeon, Korea). *E. coli* was cultivated in tryptic soy broth (Difco) at 37°C for 48 h. The culture was then centrifuged (3000 × *g* for 10 min at 4°C) using a refrigerated centrifuge (UNION 32R, Hanil Science Industrial Co., Ltd., Korea). The resulting pellet was washed twice with sterile saline (0.85%) solution and suspended in the same saline solution to achieve a viable cell density of 10^6 colony-forming units (CFU)/mL. The skin on the chicken breast was peeled off using a sterilized knife. The agar plates and the skin side of the raw chicken breast (15 × 15 × 5 mm) were inoculated with 25 and 50 μL of this solution and were spread, respectively. After that, a half of each chicken breast was kept at room temperature on a clean bench for 60 min (dry state). The other half of each chicken breast was kept for 5 min (wet state) under the same conditions and immediately treated with plasma for comparisons with the former. In order to see the difference of microbial concentration, different dilutions, which ranged from 10^3 to 10^7 CFU/mL, with saline solution were prepared and 50 μL of each solution was inoculated onto skin-off chicken breast, and then spread.

Treatment with APP jets

The APP jet device used in this study was based on a cold arc plasma with a cylindrical powered electrode with a sharpened tip, while the diameter of the emit hole was 1.5 mm. This electrode was covered with a grounded metal cathode nozzle with a cooling system (Fig. 1). The samples were treated at 50 W, and N_2 (99.9%) with total 6 standard liters per minute was used to discharge the plasma. The gas composition was changed by adding 10 standard cubic centimeters per minute (sccm) of O_2, and the distance between the sample and the APP jet was tested to obtain the optimum

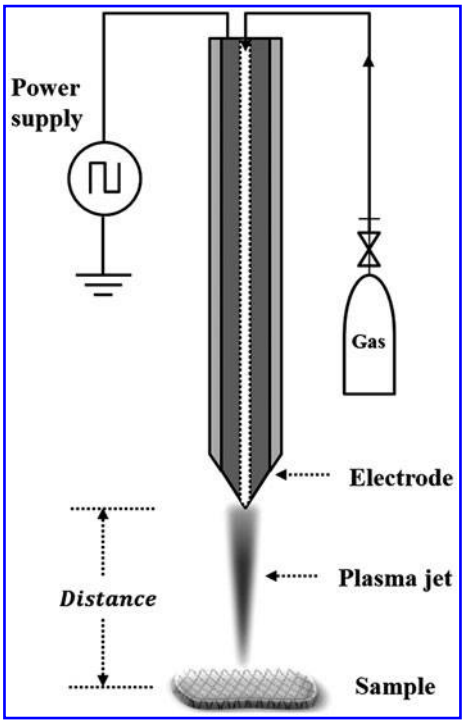

FIG. 1. Schematic diagram of the experimental setup of the atmospheric pressure plasma jet.

Table 1. Effect of Atmospheric Pressure Plasma Jets on Inactivation of *Escherichia coli* (Reduction of Log Colony-Forming Units/mL) in Agar Plates

Distance (mm)	*Time (min)*	*Gas composition*[g]	
		N_2	N_2+O_2
10	5	$0.96^{d,y} \pm 0.007$*	$1.12^{d,x} \pm 0.006$
	10	$1.28^{b,y} \pm 0.010$	$1.54^{b,x} \pm 0.020$
20	5	$1.08^{c,y} \pm 0.009$	$1.28^{c,x} \pm 0.030$
	10	$2.24^{a,y} \pm 0.008$	$2.76^{a,x} \pm 0.067$
30	5	$0.09^{f,y} \pm 0.002$	$0.20^{f,x} \pm 0.022$
	10	$0.25^{e,y} \pm 0.004$	$0.51^{e,x} \pm 0.050$

*Means ± standard error ($n=3$).
[a–f]Different letters within same column differ significantly ($p<0.05$).
[x,y]Different letters within same row differ significantly ($p<0.05$).
[g]Gas flow rate: 6 L/minute for N_2; 10 standard cubic centimeters per minute of O_2 was added for N_2+O_2.

conditions for the initial microbial inactivation efficiency. Once the optimum treatment distance and gas composition were determined, the chicken breast sample was treated at a distance of 20 mm with a N_2+O_2 in gas combination. Chicken breast at dry or wet state was treated for 5 or 10 min. The samples at dry state were divided into two groups, one-side or both-side treatments. A sterilized pincette was used to invert the chicken breast after treatment on one side was completed, in order to expose both sides to the plasma treatment. The inoculated chicken breast with different concentrations of *E. coli* was treated by the plasma jet for 5 min.

The visible emission spectrum of the APP jet was obtained through spectrometers (MAYA2000 Pro) with the relevant optical setups (Kim *et al*., 2013).

Microbial analysis

Immediately after plasma treatment, each chicken breast (2.5 g) was homogenized with 22.5 mL of sterile saline (0.85%) solution. The solution was then serially diluted in sterile saline, and each diluent (0.1 mL) was spread onto tryptic soy agar (Difco). The plates were incubated at 37°C for 48 h, and microbial counts were expressed as log CFU/g or log CFU/mL.

Statistical analysis

All experiments were conducted with three replicates, and the data were analyzed using one-way analysis of variance. Significant differences among mean values were identified using Student-Newman-Keul's multiple-range test with SAS software (SAS, Release 9.2, SAS Institute Inc., Cary, NC) using a confidence level of $p<0.05$.

Results and Discussion

Table 1 shows the inactivation of *E. coli* on agar plates following APP treatments. The combination of N_2+O_2 (10 sccm) and the longer treatment time (10 min) resulted in the highest inactivation of *E. coli* on the agar plate when treated at an optimum distance of 20 mm.

Reactive species are formed depending on the gas composition, and it has been reported that adding O_2 promotes the formation of reactive oxygen species (ROS) in the plasma, which plays the most important role in the inactivation of microorganisms among the various reactive species (Deng *et al*., 2006; Lee *et al*., 2006; Lee *et al*., 2012). As shown in Figure 2, the reactive O I, OH, and NO emissions were enhanced in the gas treatment by combination of N_2+O_2. ROS are dominant biocidal agents capable of accessing and directly attacking the cell wall (Lee *et al*., 2006; Machala *et al*., 2010). Lee *et al*. (2006) and Jung *et al*. (2010) observed morphological changes in *E. coli*, yeast, and *Bacillus subtilis* spores when they were exposed to cold plasma producing ROS as a main cause of inactivation. Enhancing the production of ROS seems to be more effective in causing inactivation, although this is not a simple task (Deng *et al*., 2006). The addition of a certain amount of oxygen in the background gas increased the effect of the plasma compared with the addition of more or less oxygen (Philip *et al*., 2002). Gweon

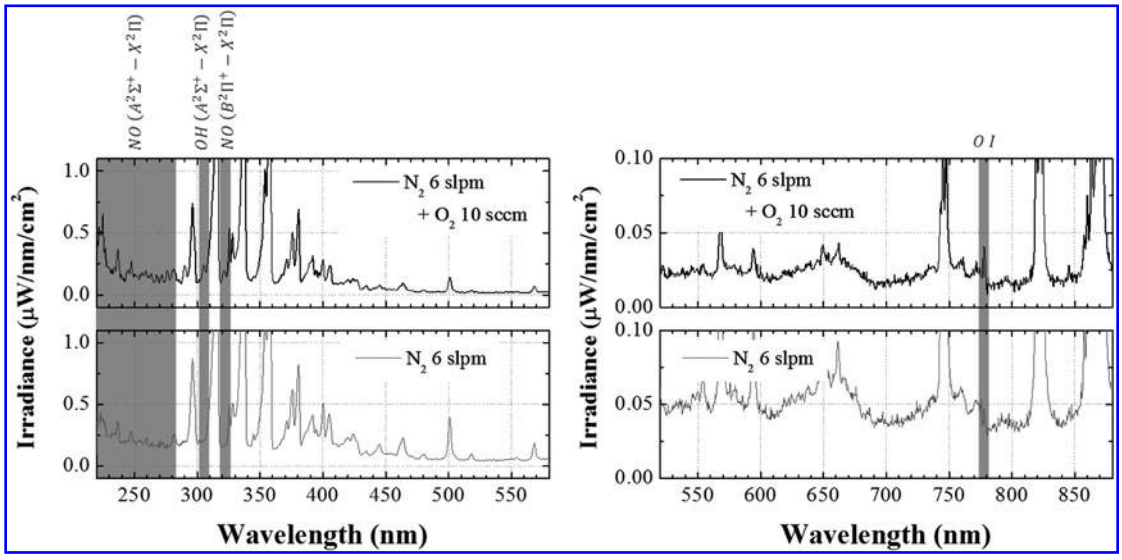

FIG. 2. Emission spectrum of the atmospheric pressure plasma jet with and without oxygen addition in gas flow. slpm, standard liters per minute; sccm, standard cubic centimers per minute.

et al. (2009) also demonstrated that a specific level of O_2 increased the plasma inactivation effect. This level was 1% when combined with He. Liu *et al*. (2008) reported that oxygen radicals can produce lipid oxidation byproducts including hexanal and malondialdehyde. However, there were no significant differences on the pH levels and lipid oxidation values of bacon and cooked egg white after APP treatment with different gas compositions (He or $He + O_2$) (Kim *et al.,* 2011; Lee *et al.*, 2012). Cooked egg white also shows no significant differences in sensory evaluation among gas compositions. However, cooked egg yolk showed significantly lower scores in odor and overall acceptability when O_2 was added (Lee *et al.*, 2012).

In principle, plasma inactivation is caused by charged particles, reactive species, UV photons, and heat (Feng *et al*., 2009). However, recently, UV photons have been reported to play a negligible role in APP (Deng *et al*., 2006; Machala *et al*., 2010; Maisch *et al*., 2012) and, in the present experiment, heat was not a possible inactivation factor because the gas temperature was near room temperature. The gas temperature here denotes the temperature of heavy particles in the plasma that is the average kinetic energy of the gas molecules in the treatment region. Charged particles and reactive species are formed in different locations (Deng *et al*., 2006). The effect of the charged particles is thought to be negligible due to their short collision mean free paths, meaning significant drop of the charged particle density even just a few millimeters away from the plasma region (Fridman *et al*., 2007). On the other hand, the chemically reactive radicals such as ROS or reactive nitrogen species can have a much longer mean free path. When comparing the reduced numbers of *E. coli* at 10- and 20-mm treatment distances as shown in Table 1, it may be suggested that the radicals have a greater inactivation capability than the charged particles. A distance of 30 mm seems too far for plasma inactivation. Using jet-type plasma, Fernández *et al*. (2012) observed that charged particles did not play a major role in the inactivation of *Salmonella*. Regardless of the decreased UV intensity and charged particle density due to the increased distance between the almond and jet plasma, a remote position was better for inactivating *E. coli* O157:H7 and *Salmonella* compared to a closer position (Niemira, 2012). Inoculated raw chicken meat and skin demonstrated that the effect of plasma is sensitive to distance between the samples and the plasma source (Noriega *et al*., 2011). In accordance with the results of Table 1, the remaining experiments were conducted with the combination of $N_2 + O_2$ (10 sccm) at a treatment distance of 20 mm.

The inactivation of *E. coli* on chicken breasts by APP jet is shown in Table 2. The condition of meat, wet or dry, had no significant effect on the inactivation of *E. coli* when one side of the samples was treated at a given treatment time. Similarly, there was no significant difference in the plasma inactivation of *L. innocua*, regardless of the moisture state of the chicken breast skins (Noriega *et al*., 2011), which was consistent with the present results. However, several other authors proposed that moisture condition affects plasma results (Noriega *et al*., 2011; Dirks *et al*., 2012; Fernández *et al*., 2012; Rød *et al*., 2012). For instance, Dirks *et al*. (2012) observed that dielectric barrier discharge plasma was more effective on a further moistened surface of chicken than other samples. In contrast, the plasma inactivation of *L. innocua* increased in samples with low moisture content, maintained in a sealed container (Rød *et al*., 2012). Table 2 further shows that the reduction of *E. coli* increased with a higher plasma exposure time, irrespective of the condition of meat with one-side treatment.

Table 2. Effect of Atmospheric Pressure Plasma Jets on Inactivation of *Escherichia coli* (Reduction of Log Colony-Forming Units [CFU]/g) in Raw Chicken Breast

Condition[x]	*Time (min)*[y]	*CFU (log/g)*
Wet	5	$1.12^{d} \pm 0.006$*
	10	$1.53^{b,c} \pm 0.017$
Dry	5	$1.14^{d} \pm 0.090$
	10	$1.76^{a,b} \pm 0.026$
	2.5 + 2.5	$1.44^{c} \pm 0.040$
	5 + 5	$1.85^{a} \pm 0.184$

*Means ± standard error ($n = 3$).
[a–d]Different letters within the column differ significantly ($p < 0.05$).
[x]Dry, plasma treatment after staying for 1 h after inoculation; wet, plasma treatment right after inoculation.
[y]2.5 + 2.5, both-sides treatment by 2.5 min each; 5 + 5, both-sides treatment by 5 min each; others: one-side treatment time.

This study is the first attempt to investigate the microbial inactivating effect of plasma when treatment is applied to both sides of the sample. The treatment for 2.5 min on each side resulted in a higher growth inhibition of *E. coli* (reduction of 1.44 log CFU/g) compared to the treatment on a single side for 5 min (reduction 1.14 log CFU/g). However, there was no significant difference between treating a single side for 10 min or both sides for 5 + 5 min. It has previously been noticed, in experiments using electron-beam irradiation, that both sides of a sample need to be treated during sterilization to overcome its low permeability (Scharf *et al*., 1999; Kang *et al*., 2012). Zhang *et al*. (1998) showed a double-sided arc plasma. However, this technique was used for welding and not for bacterial inactivation. In this experiment, the microbial inactivating potential of plasma through the treatment of both sides of samples has been proposed. However, plasma treatment on both sides of the sample for 5 + 5 min did not result in doubling the efficacy compared with a single-side treatment for 5 min. This may be because the inactivation effect of plasma does not follow a linear reduction over time (Lee *et al*., 2006; Noriega *et al*., 2011; Fernández *et al*., 2012; Lee *et al*., 2012).

Table 3. Effect of Atmospheric Pressure Plasma Jets on Inactivation of Different Concentrations of *Escherichia coli* in Raw Chicken Breasts

Microbial concentration	*Reduction*[x] *(log CFU/g)*
10^7	$1.64^{b} \pm 0.022$*
10^6	$1.62^{b} \pm 0.047$
10^5	$1.68^{b} \pm 0.014$
10^4	$1.85^{a} \pm 0.051$
10^3	$1.83^{a} \pm 0.015$

*Means ± standard error ($n = 3$).
[a,b]Different letters within the column differ significantly ($p < 0.05$).
[x]Each side of the samples was treated for 5 min.
CFU, colony-forming units.

Table 3 shows the inactivation of *E. coli* in chicken breast at different cell concentrations after the treatment of both sides with an APP jet for 5 min. The chicken breast inoculated with *E. coli* in concentrations of 10^5–10^7 presented significantly lower log reductions than the cell concentrations 10^3 and 10^4.

Several previous studies reported the relationship between initial concentration of microorganism and inactivation efficiency of plasma (Lee *et al*., 2006; Fernández and Thompson, 2011; Dirks *et al*., 2012; Fernández *et al.,* 2013). Dirks *et al.* (2012) proposed that a subpopulation of the organism was resistant to the treatment: A dielectric barrier discharge plasma treatment of raw chicken inoculated with different cell concentrations of *Campylobacter jejuni* resulted in a protective effect at the higher densities of the pathogens. Fridman *et al*. (2007) and Fernández *et al*. (2012) showed that increasing the pathogen concentration resulted in a reduction in the efficiency of inactivation by APP, which is in agreement with the findings of the present study. Fernández *et al*. (2011) proposed that multilayered cellular structures can be made when cell densities are high. In this structure, the upper layers have an effect on those beneath. In case of Lee *et al*. (2006), however, microbial inactivation of *Bacillus* spores by APP did not depend on the initial cell concentration.

In general, *E. coli* detected in chicken is 2 log, which is lower than the concentration of *E. coli* used in this study (Jang *et al*., 2008; Kim *et al*., 2009). In this respect, it is expected to be more effective in real-life practice. In addition, it may be suggested that the early stages of contaminated products are better for APP inactivation, because low cell concentrations of *E. coli* were greatly reduced by the APP jet.

Conclusions

Various APP jet parameters affect the inactivation efficiency of *E. coli* in raw chicken breasts. In the present study, the optimum condition was determined as the treatment distance of 20 mm and longer treatment time (10 min) with the addition of oxygen to the nitrogen gas flow. Furthermore, the cell concentration of sample was an important parameter for the efficacy of the inactivation process.

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Disclosure Statement

No competing financial interests exist.

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