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Effect of Inactivating *Salmonella* Typhimurium in Raw Chicken Breast and Pork Loin Using an Atmospheric Pressure Plasma Jet

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ABSTRACT

The optimal conditions for applications of an atmospheric pressure plasma (APP) jet for the inactivation of *Salmonella* Typhimurium in chicken breast and pork loins were investigated. APP jet treatment for 10 min (versus 5 minutes) showed a higher inactivation of *S.* Typhimurium in an agar plate, with the best effect at a distance of 20 mm. A treatment on both sides (both-side treatment) for 2.5 + 2.5 min showed a greater inhibition on *S.* Typhimurium growth compared to single-side treatment for 5 min, with reduction levels of 0.66 log CFU/g in chicken breast and 1.33 log CFU/g in pork loin, respectively. However, there was no significant difference between single-side treatment for 10 min and both-side treatment for 5 + 5 min in chicken breasts and pork loin samples. In conclusion, APP jet treatment conditions, including distance, time, and direction, may affect the inactivation efficiency of *S.* Typhimurium. In this experiment, distance of 20 mm and both-side treatment were the best conditions. Therefore, the optimal APP jet treatment conditions were evaluated to maximize its practical efficiency.
(**Key words :** Atmospheric pressure plasma jet, Chicken breast, Pork loin, *Salmonella* Typhimurium, Inactivation)

INTRODUCTION

Salmonellosis is an important foodborne disease that poses a major and unacceptable threat to public health in both developed and developing countries (EFSA, 2010). The Scientific Institute of Public Health reported 3,693 cases of human salmonellosis in Belgium in 2006 and the most frequently isolated serotype was *S.* Typhimurium (48.5% of the isolates) (Delhalle et al., 2009). Different food sources, such as beef, broilers, and eggs, can cause human salmonellosis (ICMSF, 1996). The proportion of human salmonellosis attributable to pork has been estimated at 9~15% in Denmark and around 21% in the Netherlands (EFSA, 2008; Hald et al., 2004). Hwang et al. (2004) reported that *Salmonella* spp. was detected on 10.2% of 68 cases of pork carcass surfaces in Korea. Therefore, an effective sanitation treatment against pathogens, especially *Salmonella* spp. is needed for foods, especially meat products.

Plasma as a non-thermal inactivation technique has recently become a novel research interest in food processing (Kim et al., 2013). Plasmas are energetic gases composed of atoms, molecules, and radicals, as well as excited and charged particles (Moreau et al., 2008). Due to these species, plasma kills various microorganisms. Utilization of non-thermal plasma discharges at pressures of or near 1 atm under ambient conditions, atmospheric pressure plasma (APP), makes the decontamination process practical and inexpensive (Yun et al., 2010). Hence, the APP system has overcome previous disadvantages of low-pressure plasma and has been used as a surface modification tool in engineering, environmental, and biomedical fields, as well as food applications (Bardos et al., 2009; Lee et al., 2012a).

There are various types of APP that have been developed with their own characteristics (Dirks et al., 2012). The jet type APP is primarily used because of its stable discharge,

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low gas temperature, and high concentrations of reactive species (Walsh et al., 2006). Previous studies showed that *Listeria monocytogenes*, inoculated into livestock products such as cooked chicken breast, ham, and cooked egg white and yolk, was efficiently reduced through using an APP jet (Lee et al., 2011; Lee et al., 2012b).

However, most previous studies were limited to confirming the inactivation of microorganisms through using an APP jet. Conditions of plasma physics, including type of discharge, distance, and treatment of the sample may influence the inactivation efficiency of APP treatment (Deng et al., 2006; Fernández et al., 2013). In addition, plasma (including APP) is a surface sterilization technology with very low penetration power (Moisan et al., 2002). Previous studies demonstrated a lower inactivation efficiency for foods treated with an electron beam compared with a gamma ray, which was attributed to the difference in penetration power between the two radiation sources (Song et al., 2009; Kim et al., 2010). The one-side plasma treatment may also cause similar results to electron beam irradiation.

The objective of this study was to investigate the optimal APP jet conditions, including distance, time, and direction for the inactivation of *S.* Typhimurium. In this experiment, the efficiency of an APP jet for the inactivation of *S.* Typhimurium was tested on a model agar and meat system, including chicken breast and pork loin.

MATERIALS AND METHODS

1. Sample preparation and sterilization

Raw chicken breast and pork loin were purchased from a local market in Daejeon, Korea on the day before the experiment and stored in a refrigerator at 4°C. Prior to inoculation, the samples were sterilized using electron-beam irradiation (35 kGy at 2.5 MeV) with a linear electron beam RF accelerator (EB-Tech, Daejeon, Korea). Nutrient agar plates (50 × 10 mm; Difco, Becton Dickinson Sparks, MD, USA) were prepared.

2. Microorganisms and inoculation

S. Typhimurium (KCTC 1925) was cultivated in nutrient broth (Difco) at 37°C for 48 h. The culture was then centrifuged (3,000 rpm 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 get a viable cell density of 10^6 CFU/mL. Prepared agar plates, skin side of raw chicken breast (15 × 15 × 5 mm) and one side of pork loin (15 × 15 × 5 mm) were inoculated with 25 and 50 μL of this solution, respectively. After inoculation, chicken breast and pork loin were kept at room temperature on a clean bench for 60 min then treated with the APP jet.

3. APP jet treatment

The APP jet device used in this study was based on an arc plasma. The shape of the anode electrode was cylindrical with a sharpened tip, while the diameter of the emission hole was 1.5 mm. This electrode was covered with a cathode nozzle with a cooling system (Fig. 1). The samples were treated at 0.5 kW and N_2 (6 liters per minute (lpm)) and O_2 (10 standard cubic centimeter per minutes (sccm)) were used for discharging the plasma. Distance between the sample and APP jet also measured to check for the effect of different plasma conditions on the efficiency of microbial inactivation using agar plates. For both-side treatment, a

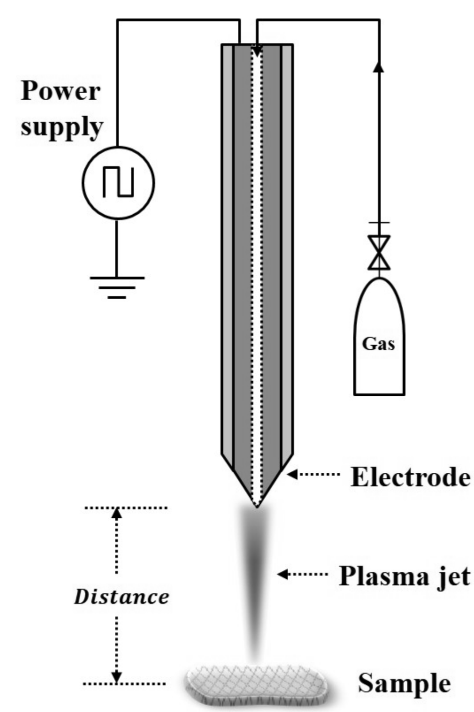

Fig. 1. Schematic diagram of the experimental set-up of the atmospheric pressure plasma jet.

sterilized pincette was used to invert the chicken breast and pork loin after one-side treatment to expose both sides to plasma.

4. Microbial analysis

After plasma treatment, the sample (2.5 g) was homogenized with 22.5 mL of sterile saline (0.85%) solution on the same day. After serial dilutions in sterile saline, each diluent (0.1 mL) was spread on nutrient agar (Difco). The plates were incubated at 37°C for 48 h, and resulting microbial counts expressed as log CFU/g and log CFU/mL.

5. Statistical analysis

All experiments in this study were performed in three individual trials (replicates) with three observations per trial. The data were analyzed using one-way analysis of variance (ANOVA), and significant differences between mean values identified using Student-Newman-Keul's multiple range test with SAS software (SAS, Release 9.2, SAS Institute Inc., Cary, NC) at a confidence level of $P < 0.05$.

RESULTS AND DISCUSSION

Plasma produces antimicrobial active species,such as ions, electrons, and UV photons, as well as reactive neutral species with sufficient energy to break covalent bonds and initiate various chemical reactions (Moisan et al., 2001). During plasma treatment, microorganisms are sterilized due to direct contact with active antimicrobial species (Moisan et al., 2002). In particular, reactive oxygen species (e.g., oxygen radicals) can produce profound effects on cells by reacting with various macromolecules (Fridovitch, 1995). Reactive oxygen species affect bacterial membrane lipids, causing the formation of unsaturated fatty acid peroxides (Lee et al., 2012). Also, the oxidation of amino acids and nucleic acids may cause changes that result in microbial death or injury (Laroussi, 2002).

S. Typhimurium on agar plates were inactivated by APP jet treatments (Table 1). The 10 min treatment showed a higher inactivation of *S.* Typhimurium, with the most effective distance at 20 mm. Niemira (2012) reported that the greatest effect observed was a 1.34 log CFU/mL reduction in *Escherichia coli* O157:H7 C9490 after a 20 s treatment at a 6 cm distance. With increasing distance from the ionizing

Table 1. Effect of the atmospheric pressure plasma jet on *Salmonella* Typhimurium inactivation on agar plates

Treatment distance (mm)	Treatment time (min)	*S.* Typhimurium population (log CFU/mL)
Untreated control		8.25 ± 0.069^{a}
10	5	7.24 ± 0.155^{b}
	10	4.86 ± 0.241^{e}
20	5	6.55 ± 0.048^{c}
	10	4.48 ± 0.091^{f}
30	5	7.38 ± 0.102^{b}
	10	5.87 ± 0.043^{d}

* Gas flow rate: 6 lpm for N_2; 10 sccm of O_2 was added for N_2+O_2.

[a−f] Different letters within same column differ significantly ($P < 0.05$).

potential across the electrodes, these extremely reactive particles tend to recombine with each other instead of the surface to be treated (Niemira and Gutsol, 2010; Ragni et al., 2010). Moisan et al. (2001) characterized the inactivation kinetics of plasma and correlated this with the physics and chemistry of the recombined plasma. Comparing the decrease in *S.* Typhimurium numbers at 10 and 20 mm distances (Table 1), the recombined plasma potentially has an increased inactivation ability compared to charged particles.

This study tested the *Salmonella* inactivation effect of plasma under both-side treatments. The both-side treatments for 2.5 min of the chicken breasts showed a higher inactivation of *S.* Typhimurium (reduction of 0.66 log CFU/g) than a single-side treatment for 5 min (reduction of 0.52 log CFU/g) (Table 2). In the pork loin, the both-side treatment for 2.5 min showed a higher inactivation of *S.* Typhimurium (reduction of 1.33 log CFU/g) compared to the single-side treatment for 5 min (reduction of 1.21 log CFU/g). However, there was no significant difference between the single-side treatment for 10 min and the both-side treatment for 5 + 5 min in the chicken breasts and pork loin samples. This might be due to a non-linear inactivation effect of plasma over time (Noriega et al., 2011; Lee et al., 2012). In practice, the both-side treatment for sterilization different food systems is used in electron beam irradiation due to their limited and low penetration power in experimental and industrial processing (Scharf and Wieszczycka, 1999 Kim et al., 2010). Our results suggest the better inactivation of *S.* Typhimurium by both-side APP jet treatment of chicken breast and pork loin.

Table 2. Effect of the atmospheric pressure plasma jet on *Salmonella* Typhimurium inactivation in chicken breast and pork loin

Treatment time (min)	*S.* Typhimurium population (log CFU/g)	
	Chicken breast	Pork loin
Control	5.66 ± 0.050[a]	6.60 ± 0.027[a]
2.5 + 2.5	5.00 ± 0.069[b]	5.27 ± 0.158[bc]
5	5.14 ± 0.026[b]	5.39 ± 0.032[b]
5 + 5	4.49 ± 0.181[c]	5.12 ± 0.065[d]
10	4.41 ± 0.046[c]	5.12 ± 0.039[cd]

* Gas flow rate: 6 lpm for N_2; 10 sccm of O_2 was added for N_2+O_2.
** Initial inoculated cell population was 8.73±0.053 log CFU/mL.
[a−f] Different letters within same column differ significantly ($P < 0.05$).

In conclusion, APP jet treatment conditions, including distance, time, and direction, may affect the efficiency of inactivating *S.* Typhimurium in chicken breast and pork loin. Therefore, optimal plasma treatment conditions should be evaluated to maximize the practical efficiency of the APP jet.

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