EFFECT OF ATMOSPHERIC PRESSURE PLASMA ON *LISTERIA MONOCYTOGES* USING DIFFERENT GAS COMPOSITIONS

B. Kim[1], H. Jung[2], W. Choe[2], Y, Jung[1], S. Jung[1], J. S. Ham[3], and C. Jo[1,*]
[1]Department of Animal Science and Biotechnology, Chungnam National University, Korea
[2]Department of Physics, Korea Advanced Institute of Science and Technology, Korea
[3]Quality Control and Utilization Division, National Institute of Animal Science, RDA, Korea

*Corresponding author (phone: +82-42-821-5774; fax: +82-42-825-9754; e-mail: cheorun@cnu.ac.kr)

***Abstract* - The aim of this study was to examine the effect of atmospheric pressure plasma (APP) on the inactivation of *Listeria monocytogenes*, an important pathogen in meat industry, when different gas compositions were applied. *L. monocytogenes* was seeded on tryptic soy agar plate of approximately 10^8 CFU/plate. The plasma was generated (90 W) using different gas compositions including He, N_2, and Ar, and with or without O_2. After exposure of APP for 1 and 2 min, the samples were incubated at 37°C for 24 h. Microbial inactivation increased with the increase of APP exposure time and the addition of O_2 in gas composition. Especially the APP with N_2/O_2 combination was the most effective and no viable cell was detected after 2 min of APP exposure. Once the optimum condition is set with further experiment, it is possible to apply the APP technology for improving the safety of meat products.**

***Index Terms* : atmospheric pressure plasma, *Listeria monocytogenes*, gas composition**

I. INTRODUCTION

Due to the present increases in outbreaks of food-borne diseases, consumers' concern has been increasing for pathogenic and spoilage microorganisms contaminated in foods. *Listeria monocytogenes* is one of the leading causes of recall in industrially processed foods and the infection of humans can result in serious health problems as well as high mortality (Posfay-Barbe & Wald, 2004). Therefore many sterilization methods to eliminate that food-borne pathogen has been suggested and used. Among them, non-thermal sterilization methods have been developed to overcome the disadvantages of thermal treatment such as side-effects in the sensory, nutritional, and functional properties of foods (Yun et al., 2010).

Plasma is a partially ionized gas by an electric discharge and comprised of ions, electrons, and ultraviolet (UV) photons, as well as reactive neutral species, atoms or radicals responsible for the destruction of microorganisms (Hury et al., 1998). Reactive oxygen species affect bacterial membrane lipids by causing the formation of unsaturated fatty acid peroxides (Moon et al., 2009). Recent development of plasma at pressures near 1 atm (atmospheric pressure plasma, APP) makes the process more practical and economical (Gweon et al., 2009). Oxidation of amino acid and nucleic acids may also cause changes that result in microbial death or injury. Song et al. (2009) and Yun et al. (2010) reported that atmospheric pressure plasma process is a promising technology to inactivate *L. monocytogenes* inoculated on sliced cheese and ham and disposable food containers, respectively. Therefore, APP technology is one of the emerging non-thermal sterilization technologies to improve food safety.

The objective of this work was to examine the effect of APP on the inactivation of *L. monocytogenes* when different gas compositions were applied and to see a further possibility of using APP as a non-thermal sterilization method for meat products.

II. MATERIALS AND METHODS

A. Preparation of microorganisms

L. monocytogenes (KCTC 3596) was obtained from a Korean Collection for Type Culture (KCTC, Daejeon, Korea). The strains were cultivated at 37°C for 18 h in a tryptic soy broth (50 mL) (Difco Laboratories, Detroit, MI, USA), and cultures of each strain was transferred aseptically to a 50 mL-centrifuge tube. *L. monocytogenes* was centrifuged (209 × *g* for 10 min at 4°C) in a refrigerated centrifuge (UNION 32R, Hanil Science Industrial, Co., Ltd., Korea). The pellet was washed twice with sterile saline (0.85%) and suspended in saline to a final concentration of approximately 10^8 CFU/mL of

the stock inoculums.

B. APP treatment

The stock inoculums of 50 μL were spread on the surface of plate count agar (PCA; Difco Laboratories, Detroit, MI, USA) plates (50 mm diameter) and allowed to completely absorb onto the agar, later stored in an ice box at 10°C before APP treatment.

A pen-type APP jet was generated at 50 kHz driving frequency and 90 W input power. The supply gases of He, Ar, N_2 were introduced for stable plasma generation with a fixed flow rate of 6 lpm (liter per minute). Furthermore, the effect of the mixture of O_2 was also investigated with or without 60 sccm (1% of lpm) of O_2 addition. For APP treatment, each plate to be treated was laid under the pen-type electrode with the plasma temperature for less than 50°C. The gap distance between the powered electrode and the treatment surface was maintained at 40 mm and the exposure times were 1 and 2 min, respectively. Samples that had not been treated were used as a control and kept at room temperature while the other samples were treated. After the APP treatment, the samples were incubated at 37°C for 24 h and microbial counts were expressed as log CFU/plate.

C. Statistical analysis

Three independent trials were conducted with 3 samples for treatment combination per each trial in the experiment. Statistical analysis was performed by one-way Analysis of Variance (ANOVA), and when significant differences were detected, the differences among the mean values were identified by Duncan's multiple range test using SAS software with the confidence level at $P < 0.05$ (SAS, Release 8.01, SAS Institute Inc., Cary, NC). Mean values and standard error of the means are reported.

III. RESULT AND DISCUSSION

Table 1 shows the inactivation of *L. monocytogenes* after the APP treatment. Microbial log reduction increased with increase of plasma exposure time. The initial population of *L. monocytogenes* was 7.59 log CFU/plate. After exposure of 1 min of APP using He, the number of viable cells was not different, but reduced to 6.72 log CFU/plate after 2 min. O_2 addition in gas composition showed higher inactivation efficiency than He only, resulting in 5.47 and 3.40 log CFU/plate after exposure for 1 and 2 min, respectively. When N_2 was used for plasma generation, approximately 4 decimal reductions were achieved after 1 min exposure and no viable cells were detected after 2 min exposure. The APP with Ar only was not as effective as He or N_2 on the inactivation of *L. monocytogenes*. However, the microbial numbers decreased about 4 and 6 log cycles for 1 and 2 min exposure, respectively, when O_2 was mixed with Ar. It has been reported that several factors can affect the inactivation efficiency of APP such as the type of microorganisms, exposure time, generation gases, and others (Song et al. 2009). Rhee et al. (2007) reported that Ar is appropriate for producing relatively large and uniform plasmas and the He plasmas usually exhibit low breakdown voltage and low gas temperature. Also, oxygen is often used in plasma for industrial applications, such as ashing and surface modification, because of its high chemical reactivity. Atmospheric pressure plasma is a partially ionized gas that is comprised of ions and electrons, as well as reactive neutral species such as radicals and excited atoms and molecules with sufficient energy to break covalent bonds and initiate various chemical reactions (Moisan et al., 2001). These reactive species or radicals induce damage to DNA as well as causing protein, lipid and membrane of microorganism damage (Montie et al., 2000, Gweon et al., 2010). The results indicate that more effective microbial reduction can be obtained when O_2 is added to gas compositions for plasma generation. It is thought that more free radicals can be produced by O_2.

IV. CONCLUSION

Our experimental results show that atmospheric pressure plasmas are applicable to inactivate *L. monocytogenes*. The inactivation efficiency is shown to be different by different gas compositions for plasma generation, and O_2 addition is more effective. Further studies with real meat or food system are needed for assuring the applicability of the technology in meat or food industry.

ACKNOWLEDGEMENT

This study was supported by Korea Rural Development Administration Fund.

REFERENCES

Gweon, B., Kim, D.B., Moon, S.Y., & Cheo, W. (2009) Escherichia coli deactivation study controlling the atmospheric pressure plasma discharge conditions. *Current Applied Physics*, 9, 625-628.

Gweon, B., Kim, D., Kim, D.B., Jung, H., Choe, W., & Shin, J.H. (2010) Plasma effects on subcellular structures. *Applied Physics Letters*, 96, 101501-1-101501-3.

Hury, S., Vidal, D.R., Desor, F., Pelletier, J., & Lagarde, T. (1998) A parametric study of destruction efficiency of *Bacillus* spores in low pressure oxygen-based plasmas. *Letters in Applied Microbiology*, 26, 417-421.

Moisan, M., Barbeau, J., Crevier, M.C., Pelletier, J., Philip, N., & Saoudi, B. (2002) Plasma sterilization. Methods and mechanisms. *Pure and Applied Chemistry*, 74, 349-358.

Montie, T.C., Kelly Wintenberg, K., & Roth, J.R. (2000) An overview of research using the one atmospheric uniform glow discharge plasma (OAUGDP) for sterilization of surfaces and materials. *IEEE Transactions on Plasma Science*, 28, 41-50.

Moon, S.Y., Kim, D.B., Choe, Gweon, B.M., Choe, W., Song, H.P., & Jo, C. (2009) Feasibility study of the sterilization on pork and human skin surface by atmospheric pressure plasmas. *Thin Solid Films*, 517, 4272-4275.

Posfay-Barbe, K.M., & Wald, E.R. (2004) Listeriosis. *Pediatrics in Review*, 25, 151-159.

Rhee, J.K., Kim, D.B., Moon, S.Y., & Choe, W. (2007) Change of the argon-based atmospheric pressure large area plasma characteristics by the helium and oxygen gas mixing. *Thin solid films*, 515, 4909-4912.

Song, H.P., Kim, B., Choe, J.H., Jung, S., Moon, S.Y., Choe, W., & Jo, C. (2009) Evaluation of atmospheric pressure plasma to improve the safety of sliced cheese and ham inoculated by 3-strain cocktail *Listeria Monocytogenes*. *Food Microbiology*, 26, 432-436.

Yun, H. J., Kim, B., J, Jung, S., Kruk, Z. A., Kim, D. B., Choe, W., & Jo, C. (2010) Inactivation of *Listeria monocytogenes* inoculated on disposable plastic tray, aluminum foil, and paper cup by atmospheric pressure plasma. *Food Control*, 21, 1182-1186.

Table 1. Inactivation of *Lsteria monocytogenes* (Log CFU/mL) inoculated on agar plate by pen-type atmospheric pressure plasma with different gas composition during storage.

Gas treatment	Exposure time (min)			SEM[2)]
	0	1	2	
		Helium gas		
Control	7.59	7.59^{x}	7.59^{x}	0.076
He	7.59^{a}	7.37^{ax}	6.72^{bxy}	0.122
He+O_2	7.59^{a}	5.47^{aby}	3.40^{by}	1.028
SEM[1)]	0.076	0.152	1.024	
		Nitrogen gas		
Control	7.59	7.59^{x}	7.59^{x}	0.076
N_2	7.59	3.31^{y}	3.33^{y}	1.375
N_2+O_2	7.59^{a}	5.48^{bxy}	ND^{cz}	0.080
SEM[1)]	0.076	0.985	0.963	
		Argon gas		
Control	7.59	7.59^{x}	7.59^{x}	0.076
Ar	7.59^{a}	5.87^{cxy}	7.04^{bx}	0.104
Ar+O_2	7.59^{a}	3.35^{aby}	1.43^{by}	1.281
SEM[1)]	0.076	0.981	0.830	

Values with different letters (a, b) within the same row differ significantly ($P < 0.05$).
Values with different letters (x, y) within the same column with the same gas use differ significantly ($P < 0.05$).
[1)] Standard error of the means (n = 9). [2)] (n = 9).