**PE4.18 Application of atmospheric pressure plasma to inactivate listeria monocytogenes inoculated on different food containers 60.00**

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***Abstract*- The objective of this study was to investigate the efficacy of atmospheric pressure plasma (APP), which is capable of operating at atmospheric pressure in air, in disposable food tray, aluminium foil, and paper cup inoculated by *Listeria monocytogenes*. The parameters considered in plasma processing were input power (75, 100, 125, and 150 W) and exposure time (60, 90, and 120 sec). The bacterial reduction in the disposable plastic tray, aluminum foil, and paper cup increased by increases of input power and exposure time of APP. The exposure times of APP required to inactivate 90% of a population ($D_{10}$ values) were calculated as 49.3, 47.7, 36.2, and 17.9 sec in disposable tray, 133.3, 111.1, 76.9, and 31.6 sec in aluminium foil, and as 526.32, 65.79, 51.81, and 41.67 sec at 75, 100, 125, and 150 W of input power, respectively. There was no viable cell detected after 90 and 120 sec of APP treatment at 150 W in disposable plastic tray. However, only 3 decimal reduction of viable cells was observed in aluminum foil and paper cup at 150 W for 120 sec. These results indicate that APP processing can be used as a method to improve the safety of food containers. However, it must be considered that there would be different effects by different materials treated.**

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***Index Terms*—Atmospheric pressure plasma, *Listeria monocytogenes*, Disposable food tray, Aluminum foil, Paper cup**

## I. INTRODUCTION

Food safety is the most critical issue for to the consumer and food industry. Contamination of foods by pathogens induces an enormous social and economical burden on health care. *Escherichia coli*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Listeria monocytogenes*, and *Enterococcus faecalis* are general food-borne pathogens that cause diseases and sometimes death for people.

Several studies suggested that various bacteria, such as *E. coli*, *S. aureus*, and *Salmonella* spp., survive not only food but also on hands, sponges, clothes, and utensils [1-3].

There are several traditional decontamination processes including thermal and non-thermal sterilization. Thermal sterilization can inactivate pathogens on produce efficiently but induces side-effects in the sensory, nutritional, and functional properties of food. To overcome these disadvantages, non-thermal sterilization methods were developed and used including chemical treatment, ultra violet, thermal treatment with vacuum, irradiation, high pressure, and others [4]. However, these processes have disadvantages which show expensive, improve equipment, residue of chemical, time-consuming and inefficient [5]. Irradiation is one of the best methods to destroy pathogenic and spoilage microorganisms among the non-thermal processes. However, this technology needs a huge facility and more consumer acceptance. High pressure processing has limitation for apply to food industry because it is operated as batch type process.

Plasma is electrically energized matter in gaseous state and can be generated by electrical discharge [6]. Prior to the mid-1990s, a stable glow discharge plasma could only be generated under vacuum or with gases such as helium and argon. However, the advance of methods for electrically generating non-thermal gas plasma at ambient conditions offers a potential new process for ensuring the microbiological safety of a

range of products [7]. The ability to generate non-thermal plasma discharges pressures at or near 1 atm makes the decontamination process practical and inexpensive. In addition, the fact that the gas temperature in such discharges remains relatively low makes their use suitable for heat-sensitive products. Atmospheric pressure plasma (APP) has been applied for the deposition, coating, synthesis, metallurgy, and etching of thin film [8]. Moreover, plasma is effective in reducing microbial populations on surface and materials such as glass, metals, and fabrics with microorganisms including bacteria, bacteria spore, yeast, and virus. Recently, Song et al. [4] reported that APP treatment was efficient for the reduction of *Listeria monocytogenes* attached on surface of materials such as sliced cheese and ham. Moon et al. [9] indicated that 4 mA conduction current and 60 °C gas temperature were obtained at input power 100 W and it can be safely applied to human skin treatment without electrical and thermal damages.

The objective of this study was to investigate the inactivation effect of *Listeria monocytogenes* inoculated on disposable plastic tray, aluminum foil, and paper cup which are usually used for food preparation.

## II. MATERIALS AND METHODS

### III.

### *Sample preparation*

Disposable plastic tray (polystyrene), aluminum foil, and paper cup (pulp) were purchased from a local market located in Daejeon, Korea at November 2008. The samples were cut up the dimensions (length x width) with 60 × 6 mm, then packed into polyethylene pouch. To sterilize the sample, 35 kGy of gamma irradiation (point source AECL, IR-79, MDS Nordion International Co. Ltd., Ottawa, ON, Canada) was performed at the Advanced Radiation Technology Institute, Jeongeup, Korea.

### *Inoculation*

Three strains of *Listeria monocytogenes* (ATCC 19114, 19115, and 19111) were obtained from a Korean Culture Center of Microorganisms (KCCM, Seoul, Korea). Each strain was cultured in a tryptic soy broth (Difco, Laboratories, Detroit, MI, USA) at 25℃ for 24 h. At stationary-phase culture of 3 strains of *L. monocytogenes* were transferred aseptically to a 50 ml centrifuge tube and were vortexed for 10 sec to ensure a homogenous cocktail. The 3-strain cocktail of each *L. monocytogenes* was centrifuged (3,000 rpm for 10 min at 4 °C) in a refrigerated centrifuge (VS-5500, Vision Scientific Co., Seoul, Korea). The pellet was washed twice with sterile saline (0.85%), and suspended in saline to a final concentration of approximately $10^9$ CFU/ml of the stock cocktail inoculum. The test culture suspension (100 μl) was uniformly and aseptically inoculated on the disposable plastic tray, aluminum foil, and paper cup, respectively. The samples were sealed in a polyethylene bag and incubated at 10 °C for 1 h to facilitate attachment of microorganisms to the samples.

### *Treatment of atmospheric pressure plasma (APP)*

The plasma generator used in the experiment producing a low temperature discharge with a dimension of 110 mm × 15 mm. The plasma generator consists of a powered rod electrode covered by a dielectric material and a grounded case. Additionally, a bottom ground electrode was placed under the powered electrode for material treatments. The electrode was powered by a 13.56 MHz rf supply through an impedance matching network. Two ground electrodes, top and bottom-electrode, were installed for an efficient processing. Helium (99.99% purity) gas with a fixed flow rate of 4 lpm (liter per minute) was introduced for stable plasma generation. The input powers in this study were 75, 100, 125, and 150 W and the exposure times were 30, 60, 90, and 120 sec. For plasma treatment, inoculated samples were placed on the bottom conductor and were in direct contact with the plasma at room temperature. The gap distance between the ground and the treatment surface was maintained at 0.6 mm. The inoculated samples without plasma treatment were also prepared as a control. After plasma treatment, the samples were immediately stored at a commercial storage condition (25 °C) and microbial analysis was performed at day 0 and 3.

### *Microbiological analysis*

Samples were vortexed with sterile saline solution (NaCl, 0.85%) for 5 min. The samples for the microbiological count were prepared in a series of decimal dilutions by a sterile saline solution. The media used for *L. monocytogenes* was tryptic soy agar (Difco, Laboratories, Detroit, MI, USA). Each diluent (100 μl) was spread in triplicate on each agar plate and the plates were incubated at 25□ for 48 h, and then the

colony formation units (CFU) per gram were calculated.

*Statistical analysis*

Three independent trials were conducted with 2 samples for treatment combination per each trial in this experiment. One-way Analysis of Variance (ANOVA) was performed, 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 errors of the mean are reported.

## IV. RESULTS AND DISCUSSION

The sensitivity of *L. monocytogenes* on the surface of disposable plastic tray, aluminum foil, and paper cup by APP are shown in Table 1. Calculated $D_{10}$ values, the exposure time required to inactivate 90% of a population, from the survival curves of 75, 100, 125, and 150 W of APP treatments were 49.26, 47.62, 36.23, and 17.99 sec in disposable plastic tray, 133.33, 111.11, 76.92 and 31.55 sec in aluminum foil, and 526.32, 65.79, 51.81, and 41.67 sec in paper cup, respectively. The $D_{10}$ value for *L. monocytogenes* was the highest when inoculated on paper cup followed by aluminum foil and disposable plastic tray by APP treatment. It suggests that the effect of plasma on different materials can be different. Nevertheless, it must be possible that APP treatment can be used to inactivate the pathogen at the surface of the food containers. Kayes et al. [10] reported that one atmosphere uniform glow discharge plasma (OAUGDP) treatment resulted $D_{10}$ values of 22, 22, and 51 sec in *S. flexneri*, *V. parahaemolyticus*, and *E. coli* O157:H7 at pH 7.0 agar, respectively. But, in agar with pH 5.0, $D_{10}$ values were 19 and 31 sec in *V. parahaemolyticus, S. enteritidis,* respectively.

The reduction of *L. monocytogenes* inoculated on disposable plastic tray, aluminium foil, and paper cup with different input powers (W) and exposure times (sec) of APP are shown in Tables 2-4. Results show that the higher input power of APP was more effective in inactivation of *L. monocytogenes* at even shorter exposure times. In the case of disposable food tray, no viable cells were detected by the APP treatment for 90 and 120 sec at 150 W. Also, APP treatment for 60 sec or longer at 125 W and for 30 sec or longer at 150 W resulted in no viable cells after 3 days of storage at 25℃. The *L. monocytogenes* inoculated on aluminum foil and paper cup shows more resistant by APP in this study. Only 3-decimal reduction was achieved by APP at 150 W for 120 sec. Furthermore, only 1-decimal reduction was observed after 3 days of storage. Ben et al. [7] observed that *E. coli* K12 inoculated on different surfaces can illustrate the importance of the surface characteristics of the substrate to microbial killing by plasma treatment. The $D_{10}$ values were detected 6, 33, and 70 sec at polypropylene, glass, and agar, respectively. The chemical environment of the cell on a given surface, its physical orientation, or its degree of surface exposure may have influenced the reduction time. APP processing has been applied for inactivation of microorganisms on the surface of nut, apple, and lettuce [11]. However, there is very limited information available on the safety enhancement of different foods, especially for meat products, and the quality of the products after APP treatment. Therefore, further studies are needed to evaluate the efficacy, limitation and applications of APP process in meat industry.

## V. CONCLUSION

The risk of food-borne pathogens contamination associated with cross-contamination depends on the level of contamination on the surfaces and the probability of its transfer to the food. Results indicated that the number of *L. monocytogenes* can be reduced by 3 to 6 logs using APP processing at 150 W for 120 sec. These results indicate that APP processing can be used as a method to improve the safety of food containers. However, it must be considered that there would be different effects by different materials. Moreover, further studies are valuable to evaluate the efficacy, limitation and applications of APP process in meat products and industry.

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Table 1. $D_{10}$ value of atmospheric pressure plasma processing on *Listeria monocytogenes* inoculated on disposable plastic tray, aluminum foil, and paper cup during storage at 25 ℃.

| Input power (W) | $D_{10}$ value[1)] | | |
|---|---|---|---|
| | Disposable food tray | Aluminum foil | Paper cup |
| 75 | 49.26 | 133.33 | 526.32 |
| 100 | 47.62 | 111.11 | 65.79 |
| 125 | 36.23 | 76.92 | 51.81 |
| 150 | 17.99 | 31.55 | 41.67 |

[1)] $D_{10}$ value is described as the time necessary to the population of cells one log or 90%. The values were determined from plots of the number of survivors versus time (sec).

Table 2. Effect of atmospheric pressure plasma processing on *Listeria monocytogenes* (log CFU/g) inoculated on disposable plastic tray during storage at 25 ℃.

| Input power (W) | Exposure time (sec) | Storage (day) | | SEM[1)] |
|---|---|---|---|---|
| | | 0 | 3 | |
| 75 | 0 | 6.79$^{aw}$ | 5.28$^{bx}$ | 0.146 |
| | 30 | 6.07$^{ax}$ | 5.11$^{bxy}$ | 0.084 |
| | 60 | 5.26$^{ay}$ | 4.58$^{by}$ | 0.195 |
| | 90 | 4.49$^{z}$ | 4.63$^{yz}$ | 0.099 |
| | 120 | 4.54$^{z}$ | 4.47$^{z}$ | 0.156 |
| | SEM[2)] | 0.165 | 0.132 | |
| 100 | 0 | 6.79$^{aw}$ | 5.28$^{bw}$ | 0.146 |
| | 30 | 5.40$^{ax}$ | 4.12$^{bxy}$ | 0.088 |
| | 60 | 4.76$^{y}$ | 4.31$^{x}$ | 0.156 |
| | 90 | 4.38$^{az}$ | 3.67$^{byz}$ | 0.152 |
| | 120 | 4.16$^{az}$ | 3.50$^{bz}$ | 0.231 |
| | SEM[2)] | 0.100 | 0.148 | |
| 125 | 0 | 6.79$^{ax}$ | 5.28$^{bx}$ | 0.146 |
| | 30 | 4.93$^{ay}$ | 3.58$^{by}$ | 0.079 |
| | 60 | 4.37$^{ay}$ | ND[3)]$^{bz}$ | 0.005 |
| | 90 | 3.57$^{az}$ | ND$^{bz}$ | 0.145 |
| | 120 | 3.33$^{az}$ | ND$^{bz}$ | 0.122 |
| | SEM[2)] | 0.173 | 0.030 | |
| 150 | 0 | 6.79$^{aw}$ | 5.28$^{bx}$ | 0.146 |
| | 30 | 4.41$^{ax}$ | ND$^{by}$ | 0.022 |
| | 60 | 3.46$^{ay}$ | ND$^{by}$ | 0.125 |
| | 90 | ND$^{z}$ | ND$^{y}$ | |
| | 120 | ND$^{z}$ | ND$^{y}$ | |
| | SEM[2)] | 0.042 | 0.020 | |

Values with different letters (a-d) within the same row differ significantly ($P < 0.05$).
Values with different letters (w-z) within the same column differ significantly ($P < 0.05$).
[1)]Standard errors of the mean (n = 6), [2)](n = 15).
[3)]Viable cell was not detected with a detection limit at $<10^1$ CFU/g.

Table 3. Effect of atmospheric pressure plasma processing on *Listeria monocytogenes* (log CFU/g) inoculated on aluminum foil during storage at 25 ℃

| Input power (W) | Exposure time (sec) | Storage (day) | | SEM[1)] |
|---|---|---|---|---|
| | | 0 | 3 | |
| 75 | 0 | $7.13^{x}$ | $6.54^{x}$ | 0.220 |
| | 30 | $6.97^{ax}$ | $6.21^{bxy}$ | 0.162 |
| | 60 | $6.86^{axy}$ | $5.92^{byz}$ | 0.076 |
| | 90 | $6.47^{ayz}$ | $5.74^{bz}$ | 0.092 |
| | 120 | $6.26^{az}$ | $5.61^{bz}$ | 0.045 |
| | SEM[2)] | 0.125 | 0.092 | |
| 100 | 0 | $7.13^{w}$ | $6.54^{x}$ | 0.220 |
| | 30 | $6.58^{x}$ | $6.16^{xy}$ | 0.134 |
| | 60 | $6.19^{ay}$ | $5.71^{byz}$ | 0.087 |
| | 90 | $6.09^{ayz}$ | $5.60^{bz}$ | 0.022 |
| | 120 | $6.02^{az}$ | $5.51^{bz}$ | 0.042 |
| | SEM[2)] | 0.039 | 0.133 | |
| 125 | 0 | $7.13^{w}$ | $6.54^{x}$ | 0.220 |
| | 30 | $6.56^{ax}$ | $5.62^{by}$ | 0.170 |
| | 60 | $6.02^{ay}$ | $5.54^{by}$ | 0.060 |
| | 90 | $5.66^{az}$ | $4.75^{bz}$ | 0.072 |
| | 120 | $5.53^{az}$ | $4.49^{bz}$ | 0.162 |
| | SEM[2)] | 0.063 | 0.144 | |
| 150 | 0 | $7.13^{w}$ | $6.54^{x}$ | 0.220 |
| | 30 | $4.89^{x}$ | $4.38^{y}$ | 0.190 |
| | 60 | $4.77^{ax}$ | $4.31^{byz}$ | 0.090 |
| | 90 | $4.51^{ay}$ | $4.13^{byz}$ | 0.110 |
| | 120 | $4.04^{z}$ | $3.73^{z}$ | 0.120 |
| | SEM[2)] | 0.069 | 0.158 | |

Values with different letters (a-d) within the same row differ significantly ($P < 0.05$).
Values with different letters (w-z) within the same column differ significantly ($P < 0.05$).
[1)]Standard errors of the mean (n = 6), [2)](n = 15).

Table 4. Effect of atmospheric pressure plasma processing on *Listeria monocytogenes* (log CFU/g) inoculated on paper cup during storage at 25 ℃.

| Input Power (W) | Exposure time (sec) | Storage (day) | | SEM[1)] |
|---|---|---|---|---|
| | | 0 | 3 | |
| 75 | 0 | 6.51 | 6.04 | 0.228 |
| | 60 | 6.31$^{a}$ | 5.34$^{b}$ | 0.060 |
| | 90 | 6.43 | 5.21 | 0.487 |
| | 120 | 6.23 | 5.28 | 0.263 |
| | SEM[2)] | 0.291 | 0.310 | |
| 100 | 0 | 6.51$^{x}$ | 6.04$^{x}$ | 0.228 |
| | 60 | 5.77$^{x}$ | 5.41$^{xy}$ | 0.296 |
| | 90 | 5.72$^{x}$ | 5.46$^{xy}$ | 0.143 |
| | 120 | 4.46$^{y}$ | 4.72$^{y}$ | 0.361 |
| | SEM[2)] | 0.276 | 0.263 | |
| 125 | 0 | 6.51$^{x}$ | 6.04$^{x}$ | 0.228 |
| | 60 | 5.36$^{y}$ | 5.13$^{y}$ | 0.045 |
| | 90 | 4.50$^{y}$ | 4.25$^{z}$ | 0.420 |
| | 120 | 4.31$^{y}$ | 4.19$^{z}$ | 0.074 |
| | SEM[2)] | 0.292 | 0.181 | |
| 150 | 0 | 6.51$^{x}$ | 6.04$^{x}$ | 0.228 |
| | 60 | 5.20$^{axy}$ | 3.41$^{by}$ | 0.215 |
| | 90 | 4.32$^{y}$ | 2.89$^{y}$ | 0.632 |
| | 120 | 3.66$^{y}$ | 2.15$^{y}$ | 0.634 |
| | SEM[2)] | 0.476 | 0.472 | |

Values with different letters (a-d) within the same row differ significantly ($P < 0.05$).
Values with different letters (w-z) within the same column differ significantly ($P < 0.05$).
[1)]Standard errors of the mean (n = 6), [2)](n = 15).