Inactivation of *Listeria monocytogenes* on agar and processed meat surfaces by atmospheric pressure plasma jets

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**A B S T R A C T**

An apparatus for generating atmospheric pressure plasma (APP) jet was used to investigate the inactivation of *Listeria monocytogenes* on the surface of agar plates and slices of cooked chicken breast and ham. He, N2 (both 7 L/min), and mixtures of each with O2 (0.07 L/min) were used to produce the plasma jets. After treatment for 2 min with APP jets of He, He + O2, N2, or N2 + O2, the numbers of *L. monocytogenes* on agar plates were reduced by 0.87, 4.19, 4.26, and 7.59 log units, respectively. Similar treatments reduced the *L. monocytogenes* inoculated onto sliced chicken breast and ham by 1.37 to 4.73 and 1.94 to 6.52 log units, respectively, according to the input gas used with the N2 + O2 mixture being the most effective. Most APP jets reduced the numbers of aerobic bacteria on the meat surfaces to <10^2 CFU/g, and the numbers remained below that level of detection after storage at 10 °C for 7 days. The results indicate that APP jets are effective for the inactivation of *L. monocytogenes* on sliced meats and for prolonging the shelf-life of such foods.

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1. Introduction

In recent years, as consumer interest in food safety has increased, many studies have been performed to identify means of securing foods against contamination with foodborne pathogens such as *Escherichia coli*, *Salmonella typhimurium*, *Staphylococcus aureus*, and *Listeria monocytogenes* (Kim et al., 2008). According to data from the Korea Food and Drug Administration, the number of foodborne diseases in 2008 increased 3.8-fold compared to the number in 2003. In the US, it was reported that foodborne pathogens result in 325,000 hospitalizations and 5000 deaths every year (WHO, 2007).

Thermal treatment has been used for many decades for the inactivation of *microorganisms*. However, its use is limited due to negative effects on the sensorial, nutritional, and functional characteristics of heat-sensitive foods (Awuah et al., 2007). Therefore, in order to develop appropriate sterilization methods without adverse changes to food quality, researchers have developed non-thermal treatments such as irradiation, high pressure processing, application of natural antimicrobials, and active packagings (Aymerich et al., 2008; Devlieghere et al., 2004).

Among recently developed non-thermal treatments, the use of low-temperature atmospheric pressure plasmas (APPs) has garnered much attention. Gas plasmas are ionized gases in a quasineutral condition. They consist of ions, electrons, and neutral particles, including atoms, molecules, radicals, and UV photons (Gweon et al., 2009; Wan et al., 2009). Many of the chemical species and UV light have been shown to be lethal toward microorganisms (Moisan et al., 2002).

APP has been used for surface modification, environmental, and biomedical applications (Bogaerts et al., 2002; Gweon et al., 2010). Although direct comparisons are not possible because of different APP systems employed for different studies, APP treatment is generally considered to be a candidate method for ensuring food safety during processing (Lee et al., 2006). Thus, Niemira and Sites (2008) applied APP to apples and observed efficient microbial inactivation with no changes in surface color or texture. Basaran et al. (2008) investigated the inactivation effects of an SF6 and air APP on different kinds of nut surfaces and found it very effective. Moon et al. (2009) reported that there was no electrical or thermal damage to pork or human skin when samples of those tissues were sterilized by He APP.

Other scientists have investigated the factors that possibly determine the inactivating effects of APP. Song et al. (2009) reported that *L. monocytogenes* inoculated on sliced cheese and ham was effectively reduced or eliminated by large area-type APP,
but input power, exposure time, and the type of food affected the efficiency of inactivation. Yun et al. (2010) obtained similar results using inoculated food containers such as plastic trays, paper cups, and aluminum foil. Further, Kim et al. (2011) and Raghi et al. (2010) found that no quality changes in bacon and eggshell occurred in response to APP treatments.

However, the application of APP to food safety improvement is still very limited. In addition, based on previous studies, there are recommendations to develop a specific APP system for food application (Song et al., 2009; Yun et al., 2010). It is also necessary to evaluate the wide range of nutritional and quality attributes of APP treated foods for obtaining the evidences and general acceptance as a food decontamination process. Therefore, the objective of this study was to evaluate the efficiency of APP jets in the inactivation of L. monocytogenes on a model agar system and real food system, including slices of chicken breast and ham.

2. Materials and methods

2.1. Sample preparation

Slices of chicken breast (Harim Co., Ltd., Iksan, Korea) and ham (CJ Co., Ltd., Jincheon, Korea) were purchased from a local market in Daejeon, Korea. Prior to the inoculation test, sliced chicken breast and ham were vacuum-packaged and sterilized by irradiation (40 kGy) using a cobalt-60 gamma irradiator at the Advanced Radiation Technology Institute, Jeongup, Korea. The agar plate used was composed of tryptic soy agar (Difco, Becton Dickinson Sparks, MD, USA).

2.2. Microorganism and inoculation

L. monocytogenes KCTC 3596 obtained from the Korean Collection for Type Culture (KCTC, Daejeon, Korea) were cultured at 37 °C for 18 h in tryptic soy broth (50 ml) (Difco Laboratories). The strain was transferred to a 50 ml centrifuge tube and centrifuged (2090× 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. The test culture suspension (10 μl) was then inoculated at 5 different points on each agar plate (50 × 10 mm), or slice of chicken breast, or ham (15 × 15 × 1 mm) and spread. To facilitate the attachment of microorganisms, the samples were left for 1 h at room temperature (approximately 22 °C).

2.3. Treatment with APP jets

A previously developed APP system was used for the study (Jung et al., 2011). Briefly, the shape of the electrode is cylindrical with a sharpened tip. The length and the diameter of the electrode are 50 mm and 5 mm, respectively. The gas flows through a 4 mm in length and 1 mm in diameter straight channel provided by the thickness of the material near the tip of nozzle. The whole length of the source is 67 mm. The electrode was powered by a 50 kHz square pulse supply (FT-Lab, HPI 500) with a 50% duty cycle.

The samples were covered by a cone-shaped glass container (Fig. 1) and treated at 2 kV peak-to-peak voltage. The agar plates seeded with L. monocytogenes were treated for 1 or 2 min, whereas the slices of chicken breast and ham were treated for 2 min. He or N2 (7 L/min) was used for discharging the plasma. In order to observe the effect of gas mixture, O2 (0.07 L/min) was added to each plasma treatment. The optimum O2 concentration for the present study was determined based on a preliminary study. For plasma treatment, inoculated samples were placed on the bottom conductor in direct contact with the plasma jets. The distance between the powered electrode and the treatment surface was maintained at 4 cm. After the treatment, the samples were stored at 10 °C and the microbial population was analyzed on Days 0 and 7.

2.4. Microbiological analysis

After treatment with APP jets, the slices of chicken breast and ham (0.5 g) were vortexed with 4.5 ml of sterile saline (0.85%) for 5 min. The samples for microbial counting were prepared in a series of decimal dilutions with sterile saline. The medium used for L. monocytogenes inoculation and total aerobic bacteria counts was tryptic soy agar (Difco). Each dilution (100 μl) was spread on triplicate plates of that medium. The plates were incubated at 37 °C for 24 h and colonies were counted. The recovery of L. monocytogenes from agar and the surface of sliced chicken breast and ham was approximately 94.9, 77.3, and 78.7%, respectively. The recovery was calculated as the percentage of the number of recovered cells divided by the number of cells in the test culture suspension.

2.5. Statistical analysis

Three different trials were carried out. Statistical analysis was performed by one-way Analysis of Variance (ANOVA), and significant differences between mean values were identified using Student-Newman-Keul’s multiple range test in SAS, Release 9.2 (SAS Institute Inc., Cary, NC) with a significance level of P < 0.05. Mean values and standard errors of the means (SEMs) are reported.

3. Results and discussion

Fig. 2 shows the effect of APP jets generated with different gas mixtures on the inactivation of L. monocytogenes cells seeded on agar plates. The numbers of L. monocytogenes were reduced by 0.87, 4.19, 4.26, and 7.59 log units after 2 min exposure with APP jets of He, He + O2, N2, or N2 + O2, respectively. The D-values, calculated
from those reductions, of APP jets against L. monocytogenes were 135.19, 21.83, 21.70, and 7.72 s using He, He + O2, N2, or N2 + O2, respectively. Song et al. (2009) reported that the D-values of a three strain cocktail of L. monocytogenes were 71.43, 62.50, 19.65, and 17.27 s from the survival curve for sliced cheese and 476.19, 87.72, 70.92, and 63.69 s for sliced ham when 75, 100, 125, and 150 W of large area-type APP generated with He was applied. The gas combination of N2 + O2 was clearly the most effective. Similarly, Gweon et al. (2009) reported that approximately 40% higher sterilization efficiency in He + O2 treatment when compared with He alone; Marsili et al. (2002) reported that O2 addition yields more radicals based on O2 and ozone during APP treatment and acts as inactivation agents; and Hurry et al. (1998) reported that O2, H2O2, and CO2-based plasmas were more effective than Ar alone. Oxygen-based plasma destroys microorganisms via combustion with the oxygen atoms and oxygen-containing radicals present in the plasma (Hurry et al., 1998). Recently, Kim et al. (2011) reported that APP treatment generated by He + O2 was more effective than that generated by He alone for inactivation of E. coli, L. monocytogenes, and S. typhimurium inoculated onto a bacon surface. However, Uhmm et al. (2007) indicated that the spore-killing efficiency of the atmospheric pressure Ar + O2 jet was very sensitive to the O2 concentration in Ar. Compared to He, N2 is known to be more effective since it yields more active species, specifically N2+ and N+ groups (Naveed et al., 2006). It was also reported that higher input power and longer exposure times result in greater inactivation (Song et al., 2009; Yun et al., 2010), as found in this study.

Fig. 2 shows microbial reduction of L. monocytogenes on slices of chicken breast and ham after treatment of 2 min exposure with APP jets. The microbial populations on slices of chicken breast and ham were significantly reduced by 1.37–4.73 log units and 1.94–6.52 log units, respectively. As with the inoculated agar plates each gas mixed with O2 was more effective than the gas alone, and the gas combination of N2 + O2 gave the highest inactivation on L. monocytogenes on these foods.

The APP treatment was more effective in inactivating L. monocytogenes on slice of ham than chicken breast (P < 0.05), with no L. monocytogenes being recovered from the slice of ham treated with APP jets of N2 or N2 + O2. This illustrates the importance for APP decontamination of the surface characteristics of the substrate. Song et al. (2009) found that APP gave greater inactivation of microorganisms on sliced cheese was observed than on sliced ham due to different characteristics on surface. The surface of sliced cheese was considerably smoother than sliced ham. Kelly-Wintenberg et al. (1999) applied air-based APP to polypropylene, glass, and agar and found that the inactivation effect of APP was more effective on polypropylene. The various interactions between APP and material surfaces can be influenced by surface diffusion, adsorption, reaction on the surface, desorption, reflection, nucleation, growth, sputtering, implantation, surface, and near-surface damage (Ataide et al., 2003). Presumably, some or all of these factors can affect the survival of microorganisms on surfaces.

The APP system used in this study was designed to minimize the different inactivation effects caused by surface irregularities between foods. Song et al. (2009) reported that 1 decimal reduction in the number of L. monocytogenes by treatment with large area-type APP for 2 min. The APP system of present study demonstrated approximately 2 decimal reductions with He or He + O2 and over 6 decimal reductions with N2 or N2 + O2 (Fig. 3) when the same time (2 min) was treated. Therefore, it can be concluded that the present system was more effective in inactivation of L. monocytogenes on ham.

The original numbers of total aerobic bacteria were 3.17 and 2.33 log CFU/g in slices of chicken breast and ham, respectively (Table 1). APP jet treatment for 2 min decreased the microbial population on slice of chicken breast to undetectable level. Except for APP jet of He, no viable cells were detected after storage for 7 days at 10 °C. However, the reduction of aerobic bacterial number was only shown in ham treated by APP jet of N2 + O2 at Day 0. There was no difference observed in the number of microorganisms of ham between Day 0 and 7.

Table 1

<table>
<thead>
<tr>
<th>Gas treatment*</th>
<th>Chicken breast</th>
<th>SEM*</th>
<th>Ham</th>
<th>SEM*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 7</td>
<td>Day 0</td>
<td>Day 7</td>
</tr>
<tr>
<td>None</td>
<td>3.17**</td>
<td>3.43**</td>
<td>0.093</td>
<td>2.33**</td>
</tr>
<tr>
<td>He</td>
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<td>1.74&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.051</td>
<td>nd&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>He + O2</td>
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<td>nd&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.307</td>
<td>nd&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
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<td>nd&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>0.289</td>
<td>0.278</td>
<td>0.525</td>
<td>0.308</td>
</tr>
</tbody>
</table>

*Different within the same column differ significantly (p < 0.05).
†Different within the same row differ significantly (p < 0.05).
‡Gas flow rate: 7 L/min for He or N2; 0.07 L/min for O2.
§Standard error of the means (n = 15).
|| Standard error of the means (n = 6).
| Viable cells were not detected with the detection limit of <10² CFU/g.

From the results, the present APP system of the type used in this study has a potential for inactivation of *L. monocytogenes* on sliced meats and for prolonging the shelf-life of such foods. However, further development of the APP system will have to be carried out if it is to provide a commercially practicable non-thermal treatment method for the food industry.

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**References**


