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Inactivation of *Listeria monocytogenes* inoculated on disposable plastic tray, aluminum foil, and paper cup by atmospheric pressure plasma

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ABSTRACT

The objective of this study was to investigate the effect of atmospheric pressure plasma (APP) on *Listeria monocytogenes* inoculated onto disposable food containers including disposable plastic trays, aluminum foil, and paper cups. The parameters considered in APP processing were input power (75, 100, 125, and 150 W) and exposure time (60, 90, and 120 s). The bacterial reduction in the disposable plastic trays, aluminum foil, and paper cups was associated with increased input power and exposure time of APP. The D_{10} values were calculated as 49.3, 47.7, 36.2, and 17.9 s in disposable plastic trays, 133, 111, 76.9, and 31.6 s in aluminum foil and 526, 65.8, 51.8, and 41.7 s in paper cups at 75, 100, 125, and 150 W of input power, respectively. There were no viable cells detected after 90 and 120 s of APP treatment at 150 W in disposable plastic trays. However, only three decimal reductions of viable cells were achieved in aluminum foil and paper cups at 150 W for 120 s. These results demonstrate that APP treatment is effective for inactivation of *L. monocytogenes* and applicable for disposable food containers. However, the type of material is crucial and appropriate treatment conditions should be considered for achieving satisfactory inactivation level.

1. Introduction

Food safety is the most critical issue for both consumers and the 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 severe diseases and in some cases even death. It has been estimated that in the Unites States alone food-borne diseases cause 76 million illnesses, 325,000 hospitalizations and 5000 deaths each year (Mead et al., 1999). Recent reports from the World Health Organization (WHO) have also concluded that the incidence of food-borne diseases is a growing public health problem in both developed and developing countries (WHO, 2007).

Several studies have suggested that various bacteria, such as *E. coli*, *S. aureus*, and *Salmonella.* spp., survive not only in food but also on hands, sponges, clothes, and disposable food containers (Jiang & Doyle, 1999; Kusumaningrum, Putten, Rombouts, & Beumer, 2002). In another study, survival of food-borne pathogens was detected at a level of 10^5 CFU/cm^2 on stainless steel surfaces (Kusumaningrum, Ridoldi, Hazeleger, & Beumer, 2003).

There are several traditional decontamination methods and they can be divided into thermal and non-thermal sterilization. Thermal sterilization can inactivate pathogens efficiently but induces side-effects in the sensory, nutritional, and functional properties of food. Therefore, it cannot be applied to some foods or materials. Moreover, food containers may be also impacted by thermal treatment. To overcome these disadvantages, non-thermal sterilization methods were developed and used including chemical treatment (Jane, Kang, Michael, Stuart, & Valgene, 2008), ultra violet (Shama, 2007), irradiation (Aziz & Moussa, 2002), high pressure (Garcia-Gonzalez et al., 2007), etc. However, these processes also have certain disadvantages which include high costs of application, requirements for specialized equipments, generation of undesirable residues, extended processing time, and lower efficiency (Brendan & Joseph, 2008). Irradiation is known as one of the best non-thermal sterilization methods to destroy pathogenic and spoilage microorganisms but may induce side-effects such as lipid oxidation, off-flavor, and loss of nutritional and sensory quality of food. In addition, this technology needs special facilities and more consumer acceptance. High pressure processing has also been successfully applied in food processing but has limitations in batch

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size processing and affects the quality of the product (Kruk et al., 2009).

Plasma is an electrically energized state of matter and can be generated by electrical discharge (Bogaerts, Neyts, Gijbels, & Mullen, 2002). Utilization of non-thermal plasma discharges at pressures at or near 1 atm in the ambient condition 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. Recently, atmospheric pressure plasma (APP) has been applied to the deposition, coating, synthesis, metallurgy, and etching of thin film etc. (Gomez et al., 2009).

The highly reactive free radicals (OH, H_2O) and H_2O_2 produced during APP process are known to play a major role in the inactivation of bacteria (Gilliland & Speck, 1967; Gweon, Kim, Moon, & Choe, 2009). Reactive oxygen species affect bacterial membrane lipids by causing the formation of unsaturated fatty acid peroxides. Oxidation of amino acid and nucleic acids may also cause changes that result in microbial death or injury. Therefore, APP is introduced as a new sanitizing technology in the field of food processing.

APP processing was effective to inactivate microorganisms in hard solid foods including nuts and soft foods including apples and lettuce (Brendan & Joseph, 2008). Recently, Song et al. (2009) reported that APP was effective to inactivate *L. monocytogenes* inoculated on sliced ham and cheese. Moon et al. (2009) suggested that APP of 4 mA conduction current and 60 °C gas temperature condition was obtained at input power 100 W using helium gas and that it could be safely applied to human skin without electrical and thermal damages. However, evidence of effectiveness on inactivation of pathogens in different foods or food containers is still very limited.

The objective of this study was to investigate the inactivation effect of APP on *L. monocytogenes* inoculated on disposable plastic trays, aluminum foil, and paper cups, which are all commonly used for food preparation and serving.

2. Materials and methods

2.1. Samples preparation

Disposable plastic trays (polystyrene), aluminum foil, and paper cups (pulp) were purchased from a local market located in Daejeon, Korea in November 2008. The samples were cut into dimensions (length x width) of 60 × 6 mm, then packed into a polyethylene pouch. To sterilize the samples, 35 kGy of gamma irradiation (AECL, IR-79, MDS Nordion International Co. Ltd., Ottawa, ON, Canada) was applied at the Advanced Radiation Technology Institute, Jeongeup, Korea. The source strength was approximately 320 kCi with a dose rate of 20 kGy/h at 10 ± 0.5 °C.

2.2. Inoculation

Three strains of *L. 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 °C for 24 h. At the stationary-phase, a culture of three strains of *L. monocytogenes* were transferred aseptically to a 50 ml centrifuge tube and were vortexed for 10 s to ensure a homogenous cocktail. Then, *L. monocytogenes* were centrifuged (1950*g* 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 trays, aluminum foil, and paper cups, respectively. The samples were sealed in a polyethylene bag and incubated at 10 °C for 1 h to facilitate the attachment of microorganisms to the samples.

2.3. Treatment by atmospheric pressure plasma (APP)

The plasma generator used in the experiment was a capacitively-coupled large area system (dimensions: 110 mm × 15 mm) and consisted of a powered rod electrode covered by a dielectric material located in a grounded case, and a bottom ground electrode that was placed under the powered electrode and used as a base for material treatment (Fig. 1). The electrode was powered by a 13.56 MHz rf supply through an impedance matching network. Helium 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 s. 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 powered electrode and the treatment surface was maintained at 6 mm. Inoculated samples without plasma treatments were also prepared as a control. After plasma treatment, the samples were immediately stored at commercial storage conditions (25 °C) and microbial analysis was performed.

2.4. Microbiological analysis

After APP treatment, 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 utilizing sterile saline solution. The media used for *L. monocytogenes* was tryptic soy agar (Difco, Laboratories, Detroit, MI, USA). Each diluent (100 l) was spread in triplicate on each agar plate and the plates were incubated at 25 °C for 48 h, after which the colony formation units (CFU) per gram were calculated.

2.5. Statistical analysis

Three independent trials were conducted with two samples for treatment combination per each trial in the 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 a confidence level of $P < 0.05$ (SAS, Release 8.01, SAS Institute Inc., Cary, NC). Mean values and standard errors of the mean are reported.

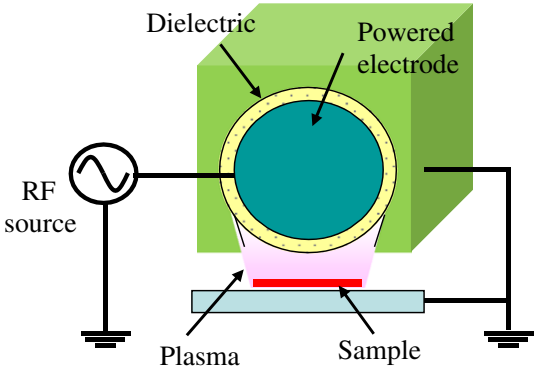

Fig. 1. Diagrammatic representation of plasma generator.