



Inactivation of foodborne pathogens on the surfaces of different packaging materials using low-pressure air plasma



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ABSTRACT

In the present study, low-pressure glow discharge plasma was used for surface decontamination of the common food packaging materials, namely glass, polyethylene, polypropylene, nylon, and paper foil. Low-pressure air plasma was generated over a vacuum pressure range of 0.5–5.0 Torr, and at a power density range of 12.4–54.1 mW/cm³. Compared to plasma-unexposed surfaces, no significant changes in optical properties, color characteristics, surface temperatures, tensile strengths, and deformation strains were observed with plasma-exposed surfaces. On plasma exposure of food pathogens-loaded packaging materials surfaces, as high as 4-log reduction (99.99%) in viable cell counts of tested food pathogens, especially *Escherichia coli* O157:H7 and *Staphylococcus aureus*, was observed within 5 min. And, the pathogens inactivation pattern can be better explained by Singh-Heldman model. Therefore, low-pressure air plasma was shown to be effective for inactivation of major foodborne pathogens, and different food packaging materials can be decontaminated using the plasma without adversely affecting their physical properties.

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1. Introduction

The most common food packaging materials are glass, wood, metal, plastics, paper and other flexible packages (Raheem, 2012). For the long-term storage of processed food without preservatives, aseptic packaging is commonly used. In aseptic packaging, raw or unprocessed product is heated, sterilized by holding at high temperature for a predetermined amount of time, then cooled and filled in sterile packages. Sterilization of packaging material is a critical step in the aseptic packaging system. Surface of packaging materials may be sterilized or decontaminated by various methods such as heat, hydrogen peroxide, irradiation, infrared light etc., and combination of these methods (Ansari & Datta, 2003). However, in recent years, plasma sterilization is emerging as an attractive substitute for chemical sterilization methods as chemicals are known for their intrinsic toxicity and leaving toxic residues on surfaces (Samuel, Matthews, & Gibson, 1988). Especially, the cold plasma sterilization methods are more suitable for microbial decontamination of heat sensitive materials (Ehlbeck et al., 2011).

Plasma can be generated in large range of temperatures and pressures by means of coupling energy to gaseous medium (Afshari & Hosseini, 2014). Plasma is ionized gas that consists of a large number of different species such as electrons, positive and negative ions, free radicals, gas molecules in the ground or excited state and quanta of electromagnetic radiation. Plasma sterilization is efficient with most gases such as O₂, N₂, air, H₂, halogens, N₂O₃, H₂O₂, CO₂, SO₂, SF₆, etc. (Ratner, Chilkotti, & Lopez, 1990). Plasma treatment can effectively inactivate a wide range of microorganisms including spores (Feichtinger, Schulz, Walker, & Schumacher, 2003; Kelly-Wintenberg et al., 1999; Lee, Paek, Ju, & Lee, 2006). The working mode of plasma sterilization is more complex; it has been hypothesized that different plasma species attack chemically the microorganisms, and may create a synergistic effect by alternating in-cell processes (Klampfl et al., 2012).

Low-temperature plasmas generated under deep vacuum (low-pressure) conditions can be used in surface modification, cleaning, decontamination and sterilization applications (Shintani, Sakudo, Burke, & McDonnell, 2010). While generating low-pressure plasma, the plasma chamber is typically evacuated below 0.1 Torr. A relatively long free path of accelerated electrons and ions is formed under such low pressures. Since these ions and neutral particles are at ambient temperatures and high voltage electrons have relatively few collisions with molecules at this pressure, the

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overall exposure conditions remain at a low temperature (Shintani et al., 2010). Although atmospheric plasmas are widely used for microbial inactivation, the lifetime of the reactive plasma species is much shorter under atmospheric conditions than that of low-pressure plasma (Shintani et al., 2010).

Bacterial inactivation effect of low-pressure, highly dissociated oxygen plasma glow on *Staphylococcus aureus* has been studied (Vujošević et al., 2011). In that study, a complete inactivation of 1.7×10^8 *S. aureus* cells within 90 s was reported. In another study, germicidal effect of oxygen plasma and nitrogen plasma has been studied using *S. aureus*, which was adsorbed on the surface of medical poly (tetrafluoroethylene) (PTFE), polyvinyl chloride (PVC) and polyethylene terephthalate (PET) films (Zhang & Chen, 2009). The study concludes that fast etching action by electrons, ions and radicals on cell membrane is the primary reason for inactivation. The effect also depends on the oxidation of gas used for discharge and the surface characteristics of material.

The Color of packaging film may influence consumer acceptability of a product (Kunte, Gennadios, Cuppett, Hanna, & Weller, 1997). Tensile properties are key indicators of the strength of a material. The possibility of change of color, appearance, tensile strength, and temperature dynamics of common food packaging materials on low-pressure air plasma treatment is unknown. Therefore, the present study aimed to determine the physical characteristics of low-pressure air plasma-exposed common food packaging materials, namely glass, polypropylene (PP), low density polyethylene (LDPE), nylon, and paper foil. This study also aimed at determining the inactivation effect or germicidal effect of low-pressure glow discharge plasma on food pathogens such as *Escherichia coli* O157:H7, *Salmonella typhimurium* and *S. aureus*, which are bound to the surface of packaging materials.

2. Materials & methods

2.1. Microorganisms

Standard cultures of food pathogens, namely *E. coli* O157:H7 ATCC 43894, *S. typhimurium* ATCC 13311, and *S. aureus* ATCC 25923 were procured from Korean Culture Center of Microorganisms (KCCM). All the strains were cultured in tryptic soy broth (TSB) (BD Company, Le Pont de Claix, France) at 37 °C for 24 h prior to use in experiments.

2.2. Packaging materials

The packaging materials were sourced from different suppliers. Glass packaging materials used in this study are glass slides (76 × 26 × 1 mm slide glass, Paul Marienfeld GmbH & Co. Lauda-Konigshofen, Germany); and the films of low density polyethylene (LDPE) (Topchemical Co., Incheon, Korea), polypropylene (PP) and nylon (Sungwon plastic packaging, Seoul, Korea), paper

foil (parchment paper) (Cleanwrap Co., Gimhae, Korea) were used as test materials.

2.3. Low-pressure air plasma generation

Low-pressure plasma generating device (CUTE series, Femto Science Co. Ltd, Hwaseong, Korea) was used. A vacuum pump was used to reduce pressure inside the plasma chamber. A schematic diagram showing the various components of low-pressure air plasma generator system is given in Fig. 1. Tank containing air as plasma-generating gas was connected to the chamber, and gas flow was regulated by turning the pressure-adjusting knob to maintain the desired partial pressure of gas within the chamber. Compressed air was pumped into the chamber that was maintained at the vacuum pressure range of 0.5–5.0 Torr, for the plasma generation. The intensity of plasma was regulated by varying the vacuum pressure and/or by varying the power density.

2.4. Determination of microbial inactivation effect of plasma

Microdrops of *E. coli* O157:H7, *S. aureus*, and *S. typhimurium* broth culture samples (10 µl each; exponential growth stage) were spotted on the surface of food packaging materials and spread (10 mm diameter circle) in such a way that they appear evenly distributed, and then dried under aseptic conditions at room temperature for 1 h. Low-pressure air plasma generation device was operated under reduced pressure conditions, and then the pathogens-loaded packaging materials were exposed to plasma. Each exposure treatment was performed over a predetermined period of time. Soon after treatment, bacteria on each film were recovered with 10 ml sterile saline solution and the number of viable cells was determined using tryptic soy agar.

2.5. Modeling of inactivation pattern

2.5.1. First-order kinetic model

Plasma inactivation pattern can be explained by a first-order kinetic model (Eq. (1)). The inactivation rate constant (k) was derived from the slope of the straight line. However, inactivation pattern can be better explained by segmented or piecewise regression analysis. Therefore, two sterilization rate constants (k_1 , k_2) were determined from slope of lines as described previously (Mok & Lee, 2012).

$$\log \frac{N_0}{N} = \frac{k}{2.303} \cdot t \quad (1)$$

Where N_0 is initial microbial population, N is microbial population at time t , t is exposure time (min), and k is inactivation rate constant.

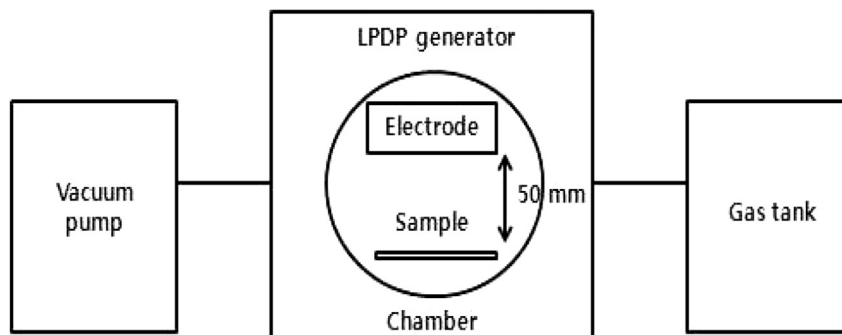


Fig. 1. Schematic diagram of low-pressure air plasma generator.

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