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Inactivation of *Staphylococcus aureus* on the beef jerky by radio-frequency atmospheric pressure plasma discharge treatment

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ABSTRACT

Radio-frequency atmospheric pressure plasma discharge as an inactivation technique was tested to reduce *Staphylococcus aureus* on the surface of polystyrene, agar, and beef jerky. *S. aureus* ATCC12600 was reduced by 3–4 log colony forming unit on the polystyrene and agar after 2 min treatment, but on beef jerky sample after 10 min treatment. It suggests that the surface feature can significantly affect the inactivation of *S. aureus* by plasma. The scanning electron microscopy analysis showed that the *S. aureus* cells were disintegrated into pieces and many holes were created. The analysis of optical emission spectrum suggests that reactive oxygen species, especially the singlet state of oxygen at 777 nm are mainly responsible for the inactivation and cellular deformation of *S. aureus*. No significant change was found in the fatty acid composition, color and shear force of the beef jerky samples ($p > 0.05$). This study shows that radio-frequency atmospheric pressure plasma is effective in inactivation of *S. aureus* on the food samples such as beef jerky with little changes in nutritional and sensory qualities.
Industrial relevance: Inactivation of *S. aureus* on polystyrene, agar, and beef jerky samples using radiofrequency atmospheric pressure plasma is presented. The plasma treatment was a useful technology to reduce a microbial contamination on the surface of thin and dehydrated food products such as beef jerky. It is promising in the industrial application since no significant change was found in the fatty acid composition, color, and shear force of the beef jerky after the plasma treatment.

1. Introduction

Thermal treatment in food processing is effective in inactivating foodborne pathogens or natural toxins with extended shelf-life. However, it negatively affects food qualities in relation to nutrient values or sensory qualities and consumes a high amount of energy (Kuldiloke & Eshtiaghi, 2008; van Boekel et al., 2010). Thus, active studies are ongoing worldwide to develop new techniques in order to effectively reduce microbial contaminants in foods with high energy efficiency without deteriorating the sensory quality (Toepfl, Mathys, Heinz, & Knorr, 2006). Among the related techniques, the interest especially in physical inactivation techniques has been increasing (Knorr, Ade-Omowaye, & Heinz, 2002; Raso & Barbosa-Cánovas, 2003). As an emerging technique, the plasma has been recently brought to the attention for the purpose although it is currently used in other industrial fields such as surface modification, lamp, or plasma displays (Bogaerts, Neyts, Gijbels, & van der Mullen, 2002; Chu, Chen, Wang, & Huang, 2002). The plasma produces highly reactive species such as free electrons, ions, radicals, excited molecules, and UV (Kaushik, Kim, Han, & Choi, 2013). Those reactive species have a microbial inactivation effect even though a disadvantage still exists such as low penetration effect. A recent development in plasma science realized a low-temperature plasma and it is considered to be useful for heat-sensitive food products. Recently a number of studies have published on the cold plasma as a non-thermal inactivation technique for foods. The plasma technique has been studied for various food items such as nuts (Basaran, Basaran-Akgul, & Oksuz, 2008; Niemira, 2012), fruits including melon and mango (Fernández, Noriega, & Thompson, 2013; Perni, Liu, Shama, & Kong, 2008; Perni, Shama, & Kong, 2008), and meats (Dirks et al., 2012; Kim et al., 2011; Song et al., 2009). Most related studies have focused on major food-borne pathogens such as *Escherichia coli*, *Salmonella*, and *Listeria monocytogenes*.

S. aureus is another bacterial agent frequently causing foodborne illnesses (Scallan et al., 2011). Even though the plasma has been studied with *S. aureus*, most studies have been focused on non-food surfaces and the data on the food items are very limited (Burts, Alexeff, Meek, & McCullers, 2009; Korachi, Gurol, & Aslan, 2010; Sureshkumar, Sankar, Mandal, & Neogi, 2010). Thus, further studies are necessary in the inactivation of *S. aureus* on food surfaces, especially considering that the surface feature of samples may greatly affect the inactivation efficiency in plasma treatment (Fernández et al., 2013). In this study, we investigated the application of radio-frequency (RF) atmospheric pressure plasma for the inactivation of *S. aureus* on the beef jerky. In

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addition, we also attempted to understand the effect of plasma on the nutritional and sensory qualities of beef jerky.

2. Materials and methods

2.1. Plasma apparatus and treatment conditions

An RF-driven atmospheric pressure plasma unit was used in this study (Fig. 1). This unit has an in-line plasma treatment system, and the length of plasma discharge is 160 mm. Argon was used as a feed gas for the generation of plasma at a fixed flow rate of 20,000 sccm with 200 W power. The powered metal electrode was placed inside the cylindrical quartz tube (150 mm long) which acts as a dielectric barrier and the plasma discharge took place between the tube and the ground electrode. Argon gas was fed from above and the generated plasma at the electrodes was pushed downward in ambient air. The temperature of plasma plume was more than 300 °C. The apparatus was equipped with a sample pedestal (15 cm × 15 cm) on which samples were placed for exposure to the plasma treatment. The distance between the plasma discharge and the samples was approximately 1 cm. A cooling pump was operated at 15 °C to circulate a coolant to prevent the discharge unit from being overheated. For uniform plasma exposure, the sample pedestal moved back and forth in a range of 6.0 cm at 1.5 cm/s in operation.

2.2. Bacterial strain and culture condition

S. aureus ATCC 12600 obtained from the Korean Culture Center of Microorganisms (KCCM, Seoul, Korea) was routinely grown on Plate Count Agar (PCA) plates at 37 °C for 1 day. An isolated colony on PCA plates was subcultured into 10 ml of Nutrient Broth (BD, Sparks, MD, USA) and incubated at 37 °C for overnight (15–16 h). An aliquot of the overnight culture was diluted by 100-fold in sterile 0.85% NaCl and the diluted cell suspension was used as an inoculum for the study.

2.3. Plasma treatment on the agar surface

Aliquots (100 μl) of the prepared inoculum were gently spread (10^6–10^7 colony forming unit, CFU) with a pipette tip on PCA plates (BD, Sparks, MD, USA) in the circle of 3.5 cm diameter. Then, the plates were semi-dried (15 min–1 h) in air at room temperature. The plates were exposed to the plasma treatment in duplicates. After the plasma treatment of the samples, the bacterial cells were suspended in 10 ml of sterile 0.85% NaCl solution from the agar plates using cell scrapers, serially diluted by 10-fold in 0.85% NaCl, and then the original suspension and each dilution were spread on PCA plates. The plates were incubated at 37 °C for approximately 24 h, and the colonies grown on the plates were enumerated to determine the number of viable cells.

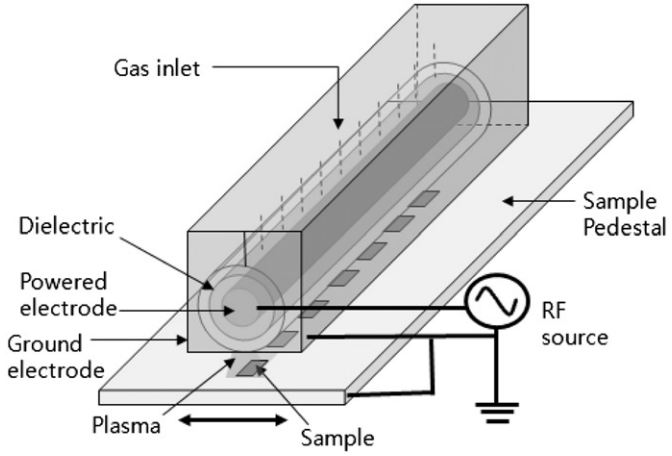

Fig. 1. Schematic diagram of radio-frequency (RF) driven atmospheric pressure plasma. The electrode was powered with radio frequency power source and covered with a quartz tube as a dielectric barrier. The plasma was generated between the dielectric barrier and ground electrode. The sample pedestal moved back and forth for uniform plasma treatment.

2.4. Plasma treatment of the beef jerky

Beef jerky samples were purchased from the markets and cut into 5 cm × 5 cm pieces. The beef jerky samples were autoclaved and placed on petri dishes covered with teflon tape and then inoculated with aliquots (100 μl) of the inoculum at 10^6–10^7 CFU. The inoculum was gently spread over the surface at 2 cm × 2 cm of the beef jerky samples with a pipette tip. After incubation at room temperature for 1–2 h, the samples were exposed to the plasma treatment for 0–10 min in duplicates. After the plasma treatment, the samples were individually placed in stomacher bags and pound in 5 ml of sterile 0.85% NaCl solution for 1 min. Then, the supernatants were serially diluted in sterile 0.85% NaCl solution by 10-fold up to 10^{-4}. The supernatants and each dilution were spread at 100 μl on PCA plates. The plates were incubated at 37 °C for 24 h, and the colonies grown on the plates were enumerated. The recovery yield for *S. aureus* of the method was around 60%. To monitor the temperature change of the beef jerky samples, the temperature probe of a thermometer (50S K/J thermometer, Fluke, Everett, WA, USA) was stuck into the sample right underneath the sample surface and the temperature was recorded every 12 s during the plasma treatment.

2.5. Thermal treatment of the beef jerky

The beef jerky samples inoculated with *S. aureus* ATCC 12600 as described above were placed in sterile and dry Whirl-Pak stomacher bags. The samples as inside the bags were submerged in water at 60 °C for 240 s, 70 °C for 300 s, or at 80 °C for 480 s to ensure the heat transfer from water to the samples. The temperature change was monitored as described above. After the treatment, the viable cells were recovered from the samples as described above.

2.6. SEM analysis

The samples were fixed in 2.5% paraformaldehyde-glutaraldehyde mixture buffered with 0.1 M phosphate (pH 7.2) for 2 h, postfixed in 1% osmium tetroxide in the same buffer for 1 h, dehydrated in graded ethanol, and substituted by isoamyl acetate. Then, they were dried at the critical point in CO_2. Finally, the samples were sputtered with gold in a sputter coater (SC502, Polaron) and observed using the scanning electron microscope, HITACHI S4300N (HITACHI, Japan) installed in the Korea Research Institute of Bioscience and Biotechnology (KRIBB).

2.7. Analysis of fatty acid composition in the beef jerky sample

The fatty acid composition of the beef jerky samples was analyzed using AOAC official method 963.22. The beef jerky samples were ground using a blender and a portion (c.a. 25 mg) was taken and mixed with 1.5 ml of 0.5 N sodium hydroxide in methanol. Then, the sample was heated on a heating block at 100 °C for 5 min, cooled, mixed with 2 ml of 14% BF_3 in methanol, and heated at 100 °C for 30 min. It was cooled to 30–40 °C, then vigorously mixed with 1 ml isooctane solution for 30 s, vigorously mixed with 5 ml saturated sodium chloride solution. After cooling, an isooctane layer was transferred to a new tube. After one more extraction with another 1 ml isooctane solution, the sample was dehydrated using sulfuric anhydride. All the steps were conducted under the condition of nitrogen gas. Fatty acid composition of the sample was analyzed with Agilent 6890 Gas Chromatograph (Hewlett-Packard). The GC was equipped with HP-FFAP capillary GC column (25 m × 0.32 mm × 0.5 μm) in which polyethylene