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Pathogen inactivation and quality changes in sliced cheddar cheese treated using flexible thin-layer dielectric barrier discharge plasma

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ABSTRACT

Cheese is recognized as a source of food-borne disease outbreaks worldwide. In this study the inactivation of pathogens on sliced cheddar cheese by using flexible thin-layer dielectric barrier discharge (DBD) plasma and its effect on food quality have been described. *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* Typhimurium populations on agar plates were significantly reduced by plasma treatment. The level of these microorganisms on sliced cheddar cheese in response to 10-min plasma treatment significantly decreased by 3.2, 2.1, and 5.8 Log CFU/g, respectively. The pH and L*-values decreased whereas thiobarbituric acid reactive substances values and b*-values increased significantly with extended exposure of the sliced cheddar cheese to DBD plasma. The total color difference (ΔE), sensory appearance and color scores showed no significant differences between DBD plasma-treated and untreated sliced cheddar cheese. However, significant reductions in flavor and overall acceptance as well as an increase in off-odor were observed. These results indicate that flexible thin-layer DBD plasma can be used to sanitize food products, but conditions should be optimized for industrial applications.

1. Introduction

With the increasing consumption of dairy products, disease outbreaks related to cheese have been reported in several countries (Koch et al., 2010; Schoder, Stessl, Szakmary-Brändle, Rossmanith, & Wagner, 2014). Makino et al. (2005) identified *Listeria monocytogenes* serotype 1/2b in washed-type cheese in February 2001, a pathogen that caused the first documented incidence of food-borne listeriosis in Japan. The commercial cheeses made from pasteurized milk between October 2006 and February 2007 also caused a massive listeriosis outbreak in Germany (Koch et al., 2010). In 2010, 41 people across five southwestern states of the United States were afflicted by food poisoning due to *Escherichia coli* O157:H7 and majority of them reported the consumption of Gouda cheese (McCollum et al., 2012). Moreover, Torres-Vitela et al. (2012) observed high incidence of *E. coli* O157:H7, *Salmonella*, *Listeria*, and staphylococci occurrence in cheeses that are commonly marketed in Mexico. Food safety is undoubtedly the important priority for the food industry as well as consumers. Thus, it is necessary to develop a reliable, cost-effective, safe, and efficient sterilization system (Korachi & Aslan, 2011; Yun et al., 2010).

In recent years, substantial efforts have been made to develop plasma-based sterilization methods. When a gas is given enough energy, the gas molecules are dissociated to form an ionized gas called plasma containing atoms, ions, electrons, and excited species (Moisan et al., 2002). Song et al. (2009) reported that the amount of 3-strain *L. monocytogenes* cocktail on cheese slices was reduced using atmospheric pressure plasma (APP) by increasing the input power and exposure time. The same plasma source was applied to bacon (Kim et al., 2011) using a helium/oxygen mixture as the process gas results in greater pathogen inactivation compared to using helium alone. Pork loins inoculated with *E. coli* and *L. monocytogenes* were treated using a DBD plasma system scanning the entire sample area, which resulted in pathogen inactivation (Kim, Yong, Park, Choe, & Jo, 2013). These studies identified the optimum conditions for pathogen sterilization and plasma system efficiency. However, plasma systems cannot be applied to packaged foods as they don't penetrate the packaging material.

Sterilization of pre-packaged food is highly desirable in the food industry, because it helps to prevent cross-contamination in comparison to a system which sterilizes each food and packaging material (Leipold, Schultz-Jensen, Kusano, Bindslev, & Jacobsen, 2011). Atmospheric pressure plasmas capable of inactivating *Candida albicans* on glass plates in sealed plastic bags have been developed (Song et al., 2012). Similarly, Rød et al. (2012) reported the reduction of *Listeria innocua* on the surface of bresaola sealed in polyethylene bags by using APP. However, these

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plasmas in the sealed package can be used with specific carrier gas like helium, oxygen, or argon. Only a few plasma types that inactivate pathogens in packaged food have been studied, and the development of APP suitable for foods remains to be a challenge. In this study, our objective was to evaluate the inactivation of pathogens on sliced cheddar cheese by using flexible thin-layer DBD plasma, and to determine the resultant quality changes.

2. Materials and methods

2.1. Sample preparation and sterilization

Sliced cheddar cheese (Seoul Milk Co., Ltd., Seoul, Korea) was purchased from a local market and cut into 15 × 15 × 2 mm sections before treating with flexible thin-layer DBD plasma. Before inoculation test with plasma treatments, a part of the cheese samples were sterilized using electron-beam irradiation (35 kGy) in a linear electron beam RF accelerator (2.5 MeV, 40 kW; EB Tech, Daejeon, Korea) to achieve the complete inactivation of the indigenous microflora. Tryptic soy agar (TSA) plates (50 × 10 mm), TSA containing 0.6% yeast extract and nutrient agar (NA) were also prepared as samples for the inoculation test (all extracts were purchased from Difco Laboratories, Detroit, USA). Cheese samples for the analysis of quality traits were not sterilized.

2.2. Microorganisms and inoculation

E. coli O157:H7 (ATCC 43894), *L. monocytogenes* (KCTC 3569) and *S.* Typhimurium (KCTC 1925) were cultivated in tryptic soy broth (Difco), tryptic soy broth containing 0.6% yeast extract, and nutrient broth (Difco) respectively, at 37 °C for 48 h. These strains were transferred to a 50 mL centrifuge tube and centrifuged at 3000 rpm for 15 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%) solution and finally suspended in the saline solution at a final concentration of approximately 10^8–10^9 CFU/mL. Aliquots of 25 and 50 μL test cultures prepared were placed at 5 different points on the surface of agar plates and the slice of cheddar cheese, respectively. To facilitate attachment of the microorganisms to agar plate and cheese samples, inoculum was spread with sterile spreader.

2.3. Treatment with flexible thin-layer DBD plasma

A flexible food-package system designed for generating DBD plasma within the food package was prepared by using the conductive layer of a commercial, zippered food package (129 × 199 mm) as the powered electrode (Fig. 1). A 0.28 mm-thick polytetrafluoroethylene (PTFE; 100 × 100 mm) sheet and a patterned conductive sheet (70 × 70 mm) were installed inside the package (Fig. 1). In addition, one of the prepared agar plates or the sliced cheddar cheese samples was placed at the bottom and the center of the package. Subsequently, the package was sealed using the zipper and a bipolar square-waveform voltage at 15 kHz was applied to the outer electrode while the inner patterned electrode was grounded. The plasma was generated at the surface of the inner electrode at 100-W peak power and 2-W average power. The carrier gas used was atmospheric gas containing nitrogen and oxygen. During thin-layer DBD plasma generation, the levels of ozone produced were measured using a UV ozone photometer (UV-H; Aeroqual Co., Auckland, New Zealand) at an absorbance of 254 nm. Agar plates inoculated with *E. coli* O157:H7, *L. monocytogenes*, and *S.* Typhimurium were treated by the flexible thin-layer DBD plasma until microorganisms were no longer detected. Sliced cheddar cheese samples inoculated with *E. coli* O157:H7, *L. monocytogenes*, and *S.* Typhimurium and non-inoculated samples were treated for 0, 2.5, 5, and 10 min.

2.4. Microbial analysis

Immediately after plasma treatment, each sliced cheddar cheese (2.5 g) was blended with 20 mL of sterile saline (0.85%) solution and then serially diluted in sterile saline. The media used for *E. coli* O157:H7, *L. monocytogenes*, and *S.* Typhimurium were TSA, TSA containing 0.6% yeast extract, and NA respectively. Each diluent (100 μL) was spread on the appropriate medium and the agar plates were incubated at 37 °C for 48 h. Plasma treated agar plates were also incubated at the same condition. All colonies were counted and the number of microorganisms was expressed as Log CFU/g or Log CFU/mL. In addition, D-value (the exposure time required to inactivate 90% of a population) was calculated using the following equation (Haas, Behsnilian, & Schubert, 1996):

$$\log\frac{N}{N_0} = -\frac{t}{D}.$$

t = time
N = the number of colonies per unit volume at time t
N_0 = the number of colonies per unit volume at time t_0 ($t_0 = 0$).

2.5. pH

After treatment with DBD plasma, 1 g of the sliced cheddar cheese was homogenized (T25 Basic, Ika Co., Staufen, Germany) with 9 mL of distilled water for 30 s (16,000 rpm). pH levels of the homogenates were measured using a pH meter (Model 750, iSTEC, Seoul, Korea) after calibration using standard buffers pH 4, 7, and 10 at room temperature.

2.6. Color measurement

Surface color measurement of sliced cheddar cheese was performed using a spectrophotometer CM 3500d (Konica Minolta

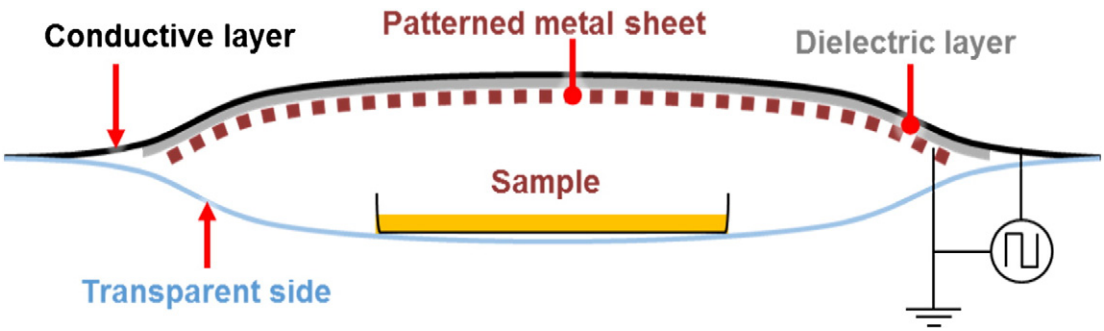

Fig. 1. Schematic diagram of the experimental setup for preparation of flexible thin-layer DBD plasma.