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Evaluation of a Dielectric Barrier Discharge Plasma System for Inactivating Pathogens on Cheese Slices

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ABSTRACT

The objective of this study was to evaluate the potential use of a dielectric barrier discharge (DBD) plasma system to improve microbial safety of sliced cheese. The atmospheric pressure plasma (APP) effect on visual appearance and a sensory evaluation were also carried out. The number of *Escherichia coli* inoculated on cheese slices decreased by 0.09, 0.47, 1.16 and 1.47 log cycles with helium (4 liters/min [lpm]) and 0.05, 0.87, 1.89 and 1.98 log cycles with He/O_2 mixture (4 lpm/15 standard cubic centimeters per minute), after being treated with plasma for 1, 5, 10, and 15 min, respectively. Significant reductions were also observed in *Staphylococcus aureus* inoculated onto cheese slices ranging from 0.05 to 0.45 log cycles with He and from 0.08 to 0.91 log cycles with He/O_2-treated samples, respectively. Adding oxygen resulted in a significant increase in inactivation of both pathogens. No visible change in the plasma-treated cheese slices was observed even though the instrumental analysis showed a significant decrease in the L*-value and an increase in the b*-value. The cheese slices were damaged after 10 and 15 min of plasma treatment. In addition, significant reductions in sensory quality including flavor, odor, and acceptability of plasma-treated cheese slices were observed. The results indicate that the DBD plasma system has potential for use in sanitizing food products, although the effect was limited. Further development of the APP system is necessary for industrial use.

(**Key words :** Atmospheric pressure plasma, Dielectric barrier discharge, Cheese slices, Pathogen inactivation, Quality)

INTRODUCTION

Plasma is a neutral ionized gas generated using an electric discharge (Tendero et al., 2006). Plasma contains photons, electrons, ions, atoms, free radicals, and excited or unexcited molecules. Due to these species, plasma kills various microorganisms (Moreau et al., 2008). Furthermore, it induces chemical reactions on material surfaces (Moisan et al., 2001). Plasma at reduced or low pressure has been used for surface processing for several decades. However, it is limited due to the vacuum system. An atmospheric pressure plasma (APP) system has been developed to overcome the disadvantage of previous low-pressure plasma (LP) and has been used as a surface modification tool in engineering, environmental and biomedical fields, and for food applications (Bardos et al., 2009; Bogaerts et al., 2002; Lee et al., 2006). The focus of recent studies was to utilize plasma on food as a nonthermal treatment. Basaran et al. (2008) and Selcuk et al. (2008) investigated the effect of LP on nuts, grains, and legumes. Perni et al. (2008), Moon et al. (2009), Song et al. (2009), Ragni et al. (2010), and Kim et al. (2011) studied the effect of APP on fruit pericarp, pork, sliced cheese and ham, shell eggs, and bacon and demonstrated the possibility of applying APP to food.

APP system effects vary depending on the conditions, such as frequency, discharge voltage, current, power, spatial uniformity, and gas temperature (Gweon et al., 2009; Kim et al., 2011). Jung et al. (2011) reported that plasma emission lines and dominant species were affected with flow transition changes. These parameters could result in different effectson APP-treated materials. In our previous studies, Song et al. (2009) and Yun et al. (2010) reported that exposure time, the nature of the gas, and surface properties affect the outcome of APP. Due to different effects from the APP discharge conditions and the surface properties of materials, it is necessary to develop an appropriate APP system for food products (Lee et al., 2008).

Dielectric barrier discharge plasma (DBD) is the most

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popular APP system. Compared to other APPs such as radio-frequency plasma, corona discharge plasma, and gliding arc discharge plasma, DBD plasma is generated between two electrodes covered with dielectric layers, so it stops electric currents and prevents streamer formation (Moreau et al., 2008). Fridman et al. (2007) found that DBD plasma minimizes the negative effects on living targets when it is used under appropriate conditions.

Therefore, this study was conducted to evaluate the potential use of a DBD system as a nonthermal treatment and to test the microbial safety of the newly developed device. The effects of APP on visual appearance and sensory evaluation of cheese were evaluated.

MATERIALS AND METHODS

1. Sample preparation

Cheese slices were purchased from a local market in Daejeon, Korea in April 2011 at 1 week intervals. The samples were cut into 15 × 15 × 2 mm sections andsterilized with UV light for 30 min prior to inoculation with *Escherichia coli* KCTC 1682 and *Staphylococcus aureus* KCTC 11764.

2. Microorganisms and inoculation

E. coli (KCTC 1682) and *S. aureus* (KCTC 11764) were obtained from the Korean Collection for Type Culture (Daejeon, Korea) and were cultured at 37°C for 18 h in tryptic soy broth (50 ml) (Difco Laboratories, Detroit, MI, USA). The strains were transferred to a 50 ml centrifuge tube and centrifuged (2,090 × g for 10 min at 4°C) in a refrigerated centrifuge (UNION 32R, Hanil Science Industrial Co., Ltd., Seoul, Korea). The pellet was then 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 inoculated into five locations on the prepared cheese slices and spread. To facilitate attachment of the microorganisms to the samples, the samples were left for 1 h at room temperature (approximately 22°C).

3. Plasma treatment

The prepared cheese slices were treated with DBD plasma produced at 3.5 kV_{pp} and a bipolar 50 kHz (low frequency range) square wave with a 50% duty cycle. Several Teflon layers, which had different numbers of holes, were sandwiched between the actuator body and the anode electrode to diffuse the carrier gas inside the actuator. The carrier gas could uniformly pass through the anode electrode made of anodized aluminum, and the discharge was generated between the anode and the ground electrode. Samples were treated under plasma for 1 to 15 min. Helium (He) gas was used to generate plasma at a fixed flow rate of 4 liter per minute. O_2 (15 standard cubic centimeters per minute, sccm) was added to the He to observe the effect of the gas mixture. The inoculated samples were placed on the bottom conductor in direct contact with the plasma for treatment. The distance between the powered electrode and the treatment surface was maintained at 5 mm.

4. Microbiological analysis

A microbiological analysis was conducted using the method of Kim et al. (2011). After the APP treatment, the cheese slices (1 g) were vortexed with 9 ml of sterile saline (0.85%) for 5 min. Microbial count samples were prepared in serial dilutions using sterile saline. The medium used for *E. coli* and *S. aureus* was tryptic soy agar. Each diluent (100 µl) was spread in triplicate, and the plates were incubated at 37°C for 24 h. The number of microorganisms was counted and expressed as log CFU/g.

5. Physicochemical analysis

(1) Surface color

The samples were placed on a round-type quartz cell (8 mm diameter), and the CIE color value was measured using a color difference meter (Spectrophotometer CM 3500d, Minolta Co., Ltd., Osaka, Japan). The instrument was calibrated to standard black and white plates before analysis. A small size aperture was used, and three measurements at different sites on each sample were averaged and used as one replication (Kim et al., 2011).

(2) Sensory evaluation

Ten panelists participated in the sensory evaluation. The panelists had at least 1 year of experience in the sensory evaluation of animal foods. The APP-treated cheese slices were cut into 30 × 30 × 2 mm sections before serving to the panelists and were evaluated using a 9-point hedonic scale

(1, extremely dislike; 5, neither dislike nor like; 9, extremely like) (Kruk et al., 2011). The parameters tested for the sensory evaluation were appearance, color, flavor, odor, texture, and acceptability. A white-colored plastic tray with a random three-digit number was used to provide the samples to each panelist, and water was provided to rinse the oral cavity during the sensory session.

6. Statistical analysis

Three different trials were carried out, and two observation numbers were obtained per trial. The statistical analysis was performed with a one-way analysis of variance, and significant differences between mean values were identified by Student-Newman-Keul's multiple range test using SAS release 9.2 software (SAS Institute Inc., Cary, NC, USA) with a confidence level of $P < 0.05$. Mean values and standard errors of the means are reported.

RESULTS AND DISCUSSION

1. Inactivation of *E. coli* and *S. aureus*

At the initial stage, the inoculated number of *E. coli* or *S. aureus* in cheese slices was 7.15 and 7.12 log CFU/g (Tables 1 and 2). After the cheese was treated with APP for 1, 5, 10, and 15 min, the number of *E. coli* decreased to 7.06, 6.68, 5.99, and 5.68 log CFU/g with He and to 7.10, 6.28, 5.26, and 5.17 log CFU/g with the He/O_2 mixture, respectively. Thus, these samples achieved significant reductions of up to 1.47 nd 1.98 log cycles for *E. coli*, and D-values were 9.80 min ($R^2 = 0.99$) and 6.85 min ($R^2 = 0.93$), respectively. *E. coli* is a Gram-negative bacterium, whereas *S. aureus* is a Gram-positive bacterium. The inactivation rate of Gram-negative bacteria by APP is superior to that of Gram-positive bacteria (Montie et al., 2000). Charged particles might have a role in rupture of the bacterial wall (Mendis et al., 2000). Those authors claimed that Gram-negative bacteria are more susceptible to membrane rupture as they have a rough and thin membrane, and that electrostatic force can overcome the tensile strength of the outer membrane. Reactive species, in particular, O_2-based reactive species and OH radicals, are important agents that cause lethal damage to cell membranes, lipids, proteins, and nucleic acids (Laroussi, 2002). That study investigated the possible role of UV radiation as well as reactive species. However, UV radiation was not the main agent inactivating microorganisms when APP was generated. Besides, there are still unknown agents and reactions that should be understood through further investigation. APP sterilization has been examined in many bacteria in the vegetative and spore states with different experimental designs. All ethese studies indicate that the discharge conditions are the most important factor for a sterilization effect. Kim et al. (2011) applied

Table 1. The effect of dielectric barrier discharge plasma on *Escherichia coli* (KCTC 1682) inoculated onto cheese slices and treated for different times (log CFU/ml)

Treatment time (min)	Input gas[1]		SEM[2]
	He	He+O_2	
0	7.15^{a}	7.15^{a}	0.037
1	7.06^{a}	7.10^{a}	0.033
5	$^{A}6.68^{b}$	$^{B}6.28^{b}$	0.029
10	$^{A}5.99^{c}$	$^{B}5.26^{c}$	0.096
15	$^{A}5.68^{d}$	$^{B}5.17^{c}$	0.071
SEM[3]	0.034	0.077	

[1] Flow rate, 4 liters He/min; O_2, 15 standard cubic centimeters per minute; input power, 3.5 kV

[2] Standard error of the mean (n = 6) [3] (n = 15).

[a–d] Different letters within the same column differ significantly ($p < 0.05$).

[A, B] Different letters within the same row differ significantly ($p < 0.05$).

Table 2. The effect of dielectric barrier discharge plasma on *Staphylococcus aureus* (KCTC 11764) inoculated onto cheese slices and treated for different times (log CFU/ml).

Treatment time (min)	Input gas[1]		SEM[2]
	He	He+O_2	
0	7.12^{a}	7.12^{a}	0.047
1	7.07^{ab}	7.04^{a}	0.015
5	$^{A}7.02^{b}$	$^{B}6.81^{b}$	0.029
10	$^{A}6.68^{c}$	$^{B}6.53^{c}$	0.033
15	$^{A}6.67^{c}$	$^{B}6.21^{d}$	0.052
SEM[3]	0.029	0.044	

[1] Flow rate, He 4 liters helium/min; O_2, 15 standard cubic centimeters per minute; input power, 3.5 kV

[2] Standard error of mean (n = 6) [3] (n = 15).

[a–d] Different letters within the same column differ significantly ($p < 0.05$).

[A, B] Different letters within the same row differ significantly ($p < 0.05$).

large area type APP with He or a He/O_2 mixture on *E. coli*, *Listeria monocytogenes*, and *Salmonella* Typhimurium inoculated on bacon and reported that APP inactivated a slightly higher number of *E. coli* than that of *L. monocytogenes*. Significant but relatively minor reductions ranged from 0.05 to 0.45 log cycles for the APP-treated samples with He and from 0.08 to 0.91 log cycles for the He/O_2 mixture of inoculated *S. aureus*, respectively. The D-values of inoculated *S. aureus* were 31.25 min ($R^2 = 0.90$) for APP treatment with He and 16.95 min for the He/O_2 mixture ($R^2 = 1.00$). Our results agreed with the observation that the APP inactivation rate was better in Gram-negative than Gram-positive bacteria. The reason that Gram-negative bacteria are inactivated at a greater rate is the cell-wall structure, as APP destroys the cell membrane leading to death (Montie et al., 2002; Korachi et al., 2009). Kostov et al. (2010) investigated the effects of APP on cell-wall structure using scanning electron microscopy and observed more intensive disruption of the cell wall of *E. coli* than that of *S. aureus* after APP treatment on agar. Those authors reported 15 and 18 min D-values for each pathogen and stated that Gram-negative bacteria were susceptible to the effect of APP due to membrane structure. Although minor reductions were revealed in both bacteria-inoculated samples, adding oxygen (15 sccm) resulted in a significantly better effect. This result suggests that adding oxygen could increase the inactivation rate, as it produces more active radicals (Gweon et al., 2009). However, previous results have shown that the D-values of a large area type plasma system against *E. coli* were 142, 76.9, and 55.6 sec and 90.9, 66.7, and 28.6 sec at 75, 100, and 125 W using He and a He/O_2 mixture, respectively, inoculated onto bacon (Kim et al., 2011). Furthermore, the number of *L. monocytogenes* on slices of chicken breast and ham decreased by 1.374.73 log cycles and 1.946.52 log cycles, respectively, after a 2 min treatment with an APP jet system and different gases (Lee et al., 2011). Therefore, the inactivation efficiency of DBD plasmain the present study was limited when compared with that of previous APP systems. Researchers have investigated how to optimize APP conditions but there are still unknown interactions between the plasma and treated samples. Miao et al. (2011) reported that plasma discharge power decreases as the distance between the electrode and sample increases. The DBD plasma used in our study has potential for use in heat-sensitive foods under appropriate conditions, but further studies are necessary.

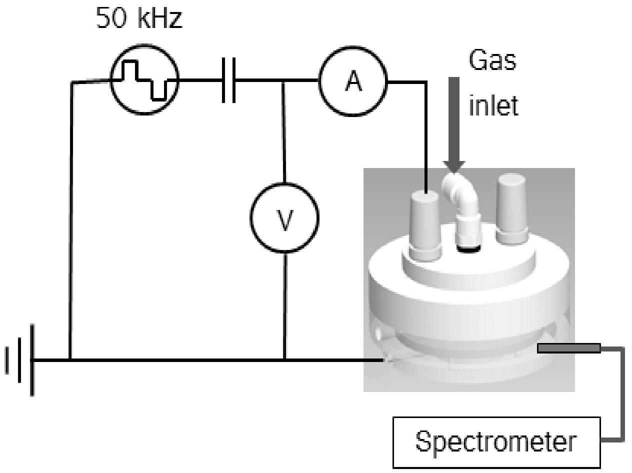

Fig. 1. Schematic diagram of the dielectric barrier discharge plasma system.

2. Physicochemical properties

APP treatment significantly decreased the L*-values (69.48 to 66.57-68.64) and increased the b*-values (34.28 to 35.84-37.75) of the cheese slices (Table 3). Cheng et al. (2010) treated various fibrous materials with APP and reported a 6.67-14.81% increase in the yellow index. They reported that the effect was induced by oxidation. Kim et al. (2011) investigated the effect of large area type APP on surface color of bacon and found a significant decrease in the L*-value after treatment. The He/O_2 treatments resulted in a significant decrease in L*-value, similar to our results. Moisture evaporation during the APP process may have induced the decrease in the L*-value. Nevertheless, no visible change was observed for the APP-treated cheese slices by sensory panelists (Table 4). Moon et al. (2009) and Kim et al. (2011) also agreed that large area type APP does not induce significant visible changes after treatment of pork, human skin, or bacon. However, the samples were damaged after 10 and 15 min of APP treatment (Figs. 2 and 3). This result may be related to the arc created during the APP process. Arc formation is useful but not appropriate for treating food products, particularly heat-sensitive materials. Further research is required to optimize the APP condition for use with food.

Significant reductions in flavor, odor, and overall acceptability of APP-treated cheese slices were observed (Table 4). In particular, APP treatments with the He/O_2 mixture resulted in the lowest score among all APP-treated samples (5.3 and 5.8 in the control vs. 2.4 and 2.3 in

Table 3. Effect of dielectric barrier discharge plasma on surface color of the cheese slices

Color value	Input gas1)	Treatment time (min)	Storage (day)			SEM2)
			0	7	14	
L* (Lightness)	None	0	$^{B}69.48^{a}$	$^{A}71.17^{a}$	$^{C}68.24^{a}$	0.266
	He	1	$^{B}68.92^{ab}$	$^{A}70.02^{b}$	$^{C}67.95^{a}$	0.226
		5	$^{A}68.64^{b}$	$^{AB}67.85^{d}$	$^{B}67.62^{ab}$	0.237
		10	$^{A}67.31^{c}$	$^{B}66.14^{f}$	$^{B}66.09^{cd}$	0.279
	He+O_2	1	$^{A}68.99^{ab}$	$^{A}69.00^{c}$	$^{B}67.51^{ab}$	0.160
		5	$^{A}67.60^{c}$	$^{AB}66.96^{e}$	$^{C}66.68^{bc}$	0.244
		10	$^{A}66.57^{d}$	$^{A}66.43^{ef}$	$^{B}65.38^{d}$	0.271
		SEM3)	0.201	0.219	0.298	
a* (Redness)	None	0	$^{B}11.13^{c}$	$^{A}11.78^{abc}$	$^{A}11.89^{ab}$	0.184
	He	1	11.71^{b}	11.43^{c}	11.68^{ab}	0.147
		5	$^{B}11.59^{bc}$	$^{AB}11.76^{abc}$	$^{A}12.09^{ab}$	0.108
		10	12.42^{a}	11.89^{a}	12.20^{a}	0.190
	He+O_2	1	$^{B}11.27^{bc}$	$^{AB}11.51^{bc}$	$^{A}11.78^{ab}$	0.138
		5	$^{A}12.36^{a}$	$^{B}11.87^{ab}$	$^{B}11.63^{b}$	0.131
		10	$^{A}12.33^{a}$	$^{B}11.88^{a}$	$^{AB}12.05^{ab}$	0.095
		SEM3)	0.160	0.110	0.162	
b* (Yellowness)	None	0	$^{B}34.28^{c}$	$^{B}34.70^{d}$	$^{A}37.11^{bc}$	0.233
	He	1	$^{A}36.00^{b}$	$^{B}34.12^{d}$	$^{A}36.85^{c}$	0.363
		5	36.06^{b}	36.65^{bc}	36.84^{c}	0.427
		10	37.75^{a}	37.66^{ab}	38.30^{a}	0.392
	He+O_2	1	$^{C}34.65^{c}$	$^{B}35.73^{c}$	$^{A}37.15^{abc}$	0.284
		5	$^{B}35.84^{b}$	$^{A}37.56^{ab}$	$^{A}37.44^{abc}$	0.277
		10	$^{B}36.03^{b}$	$^{A}37.71^{a}$	$^{A}38.21^{ab}$	0.221
		SEM3)	0.288	0.320	0.356	

1) Flow rate, 4 liters helium/min; O_2, 15 standard cubic centimeters per minute; input power, 3.5 kV

2) Standard error of the mean (n = 15) 3)(n = 21) .

$^{a-f}$ Different letters within the same column in different gas treatments differ significantly ($p < 0.05$).

$^{A-C}$ Different letters within the same row differ significantly ($p < 0.05$).

plasma-treated cheese slice using the He/O_2 mixture for 10 min for flavor and odor, respectively). A newly developed plasma jet system in our laboratory was applied to cooked egg white and yolk. The APP jet did not affect sensory quality of the cooked egg white but affected flavor, taste, and acceptability of the cooked egg yolk, and adding oxygen resulted in a worse score than He or nitrogen generated alone (data not shown). It is well known that the lipid concentration of egg white is considerably lower than that of egg yolk. Liu et al. (2008) reported that oxygen radicals produce lipid oxidation byproducts such as hexanal and malondialdehyde. Due to the high fat concentration in cheese slices, the higher amount of lipid oxidation byproducts produced may induce off-odor in APP-treated samples. However, the thiobarbituric acid reactive substances values of APP treated-bacon (Kim et al., 2011) and the cooked egg white and yolk (data not shown) were not different. It is necessary to identify what affects the sensory quality of APP-treated foods so that we can prevent negative effects and develop APP as a nonthermal treatment to improve food

Table 4. Effect of dielectric barrier discharge plasma on sensory evaluation of the cheese slices

Input gas[1)]	Time treatment (min)	Parameters					
		Appearance	Color	Flavor	Odor	Texture	Acceptability
None	0	5.5	5.4	5.3^{a}	5.8^{a}	4.7	5.3^{a}
He	1	5.5	5.6	4.1^{b}	4.1^{b}	4.7	3.8^{b}
	5	5.4	5.4	4.7^{ab}	4.7^{b}	4.9	4.4^{b}
	10	5.0	5.3	4.5^{ab}	4.8^{ab}	5.1	4.4^{b}
He+O_2	1	5.4	5.5	3.0^{c}	2.7^{c}	4.6	2.5^{c}
	5	4.7	5.1	2.7^{c}	2.1^{c}	4.6	1.9^{c}
	10	4.4	5.0	2.4^{c}	2.3^{c}	4.6	2.0^{c}
	SEM[2)]	0.39	0.45	0.32	0.37	0.28	0.29

[1)] Flow rate, 4 liters helium/min; O_2, 15 standard cubic centimeters per minute; input power, 3.5 kV

[2)] Standard error of the mean (n = 21).

[a-c] Different letters within the same column in the different gas treatments differ significantly ($p < 0.05$).

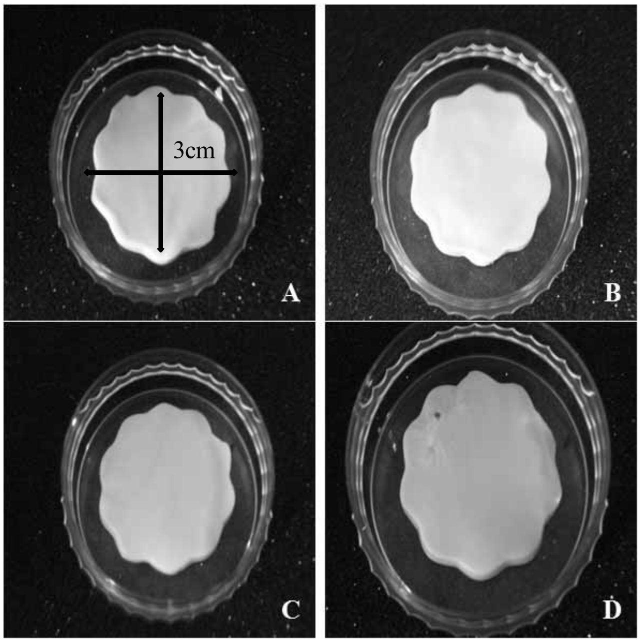

Fig. 2. Surface appearance of a cheese slice after dielectric barrier discharge plasma treatment with helium for 1 (A), 5 (B), 10 (C), and 15 min (D).

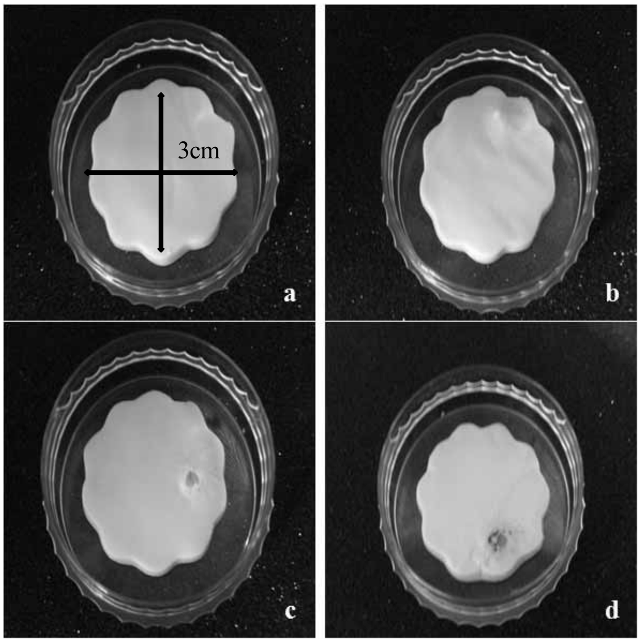

Fig. 3. Surface appearance of a cheese slice after dielectric barrier discharge plasma treatment with a helium/oxygenmixture for 1 (A), 5 (B), 10 (C), and 15 min (D).

safety.

APP is one of the promising methods for improving food safety. However, the present DBD plasma system must be developed further with higher pathogen inactivation efficiency and minimum adverse effects on sensory quality.

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