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

Hae In Yong [a], Hyun-Joo Kim [a], Sanghoo Park [b], Kijung Kim [b], Wonho Choe [b], Suk Jae Yoo [c], Cheorun Jo [a,*]

[a] Department of Agricultural Biotechnology, Center for Food and Bioconvergence, Research Institute of Agriculture and Life Science, Seoul National University, Seoul 151-921, Republic of Korea
[b] Department of Physics, Korea Advanced Institute of Science and Technology, Daejeon 305-701, Republic of Korea
[c] Plasma Technology Research Center, National Fusion Research Institute, Gunsan 573-540, Republic of Korea

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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

* Corresponding author at: Department of Agricultural Biotechnology, Seoul National University, Seoul 151-921, Republic of Korea. Tel.: +82 2 880 4804; fax: +82 2 873 2271.
E-mail address: cheorun@snu.ac.kr (C. Jo).

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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

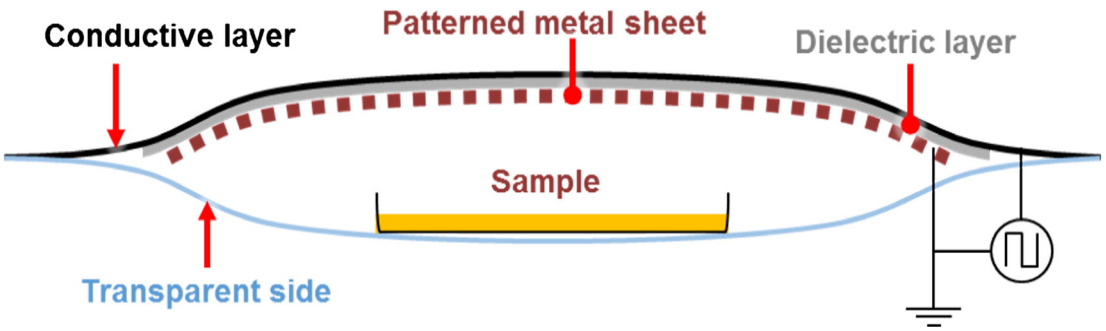

Fig. 1. Schematic diagram of the experimental setup for preparation of flexible thin-layer DBD plasma.

Censing Inc., Japan), and Hunter color values, L* (lightness), a* (redness), and b* (yellowness), were determined. The instrument was calibrated to a standard black and white plate before analysis. The Hunter values were monitored by a computerized system using spectra magic software (Konica Minolta Sensing, Inc.). The total color difference (ΔE) for each sample was calculated using the following equation:

$$\Delta E = \sqrt{(\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2}.$$

2.7. Thiobarbituric acid reactive substance (TBARS) values

Lipid oxidation was determined by calculating 2-thiobarbituric acid reactive substance (TBARS) values according to the method by Jung et al. (2011). Each sample (3 g) was added to 9 mL of distilled water and 50 μL of butylated hydroxy toluen (7.2% in ethanol) in a centrifuge tube (50 mL) and homogenized (16,000 rpm) for 30 s. The homogenate (1 mL) was transferred to a centrifuge tube (15 mL) and added to 2 mL of thiobarbituric acid (TBA)/trichloroacetic acid (TCA) solution (20 mM TBA in 15% TCA). The tubes were heated in a water bath at 90 °C for 30 min, cooled in cold water and then centrifuged at 3000 rpm for 10 min. The absorbance of the supernatant was measured at 532 nm using a spectrophotometer (DU 530, Beckman Instruments Inc., Fullerton, CA, USA) and lipid oxidation was reported as mg malondialdehyde per kg sample.

2.8. Sensory evaluation

Sensory evaluation of slice cheddar cheese samples was performed by a ten-member panel who has experience in sensory analysis of animal product for at least one year. One slice of cheddar cheese sample (15 × 15 × 2 mm) was cut into 4 pieces and one piece was provided to one panelist for evaluating appearance, color, flavor, taste, off-odor, and overall acceptance. The sample was scored on a 1- (extremely dislike) to 9-point (extremely like) scale, except for off-odor evaluation wherein a high score was given with increasing off-odor.

2.9. Statistical analysis

All experimental procedures were conducted in triplicate with 2 observation numbers except for sensory analysis (10 observations). Statistical analysis was performed by one-way analysis of variance (ANOVA), and significant differences between mean values were identified using Duncan's multiple comparison test in SAS software (SAS, Release 9.2, SAS Institute Inc., Cary, NC) with a significance level of $p < 0.05$.

3. Results and discussion

3.1. Inactivation of food-borne pathogens

Agar plates were placed at the bottom of the packaging material and exposed to flexible thin-layer DBD plasma. The numbers of *E. coli* O157:H7, *L. monocytogenes*, and *S.* Typhimurium on the agar plates were initially 8.9, 10.3, and 7.5 Log CFU/mL, respectively, which reduced significantly to 5.9, 7.6, and 6.2 Log CFU/mL when exposed for 3 min, 7 min, and 60 s of flexible thin-layer DBD plasma treatment, respectively. No viable *E. coli* O157:H7, *L. monocytogenes*, and *S.* Typhimurium cells were detected after the treatment for 4 min, 10 min, and 75 s, respectively (Fig. 2). In general, plasma sterilization works via three basic mechanisms (Koch et al., 2010; Laroussi, 2005; Moisan et al., 2002): direct breakage of DNA by ultraviolet (UV) photons; destruction of cell membranes by charged particles (e.g., O^-, OH^-, H^+, and e^-); and

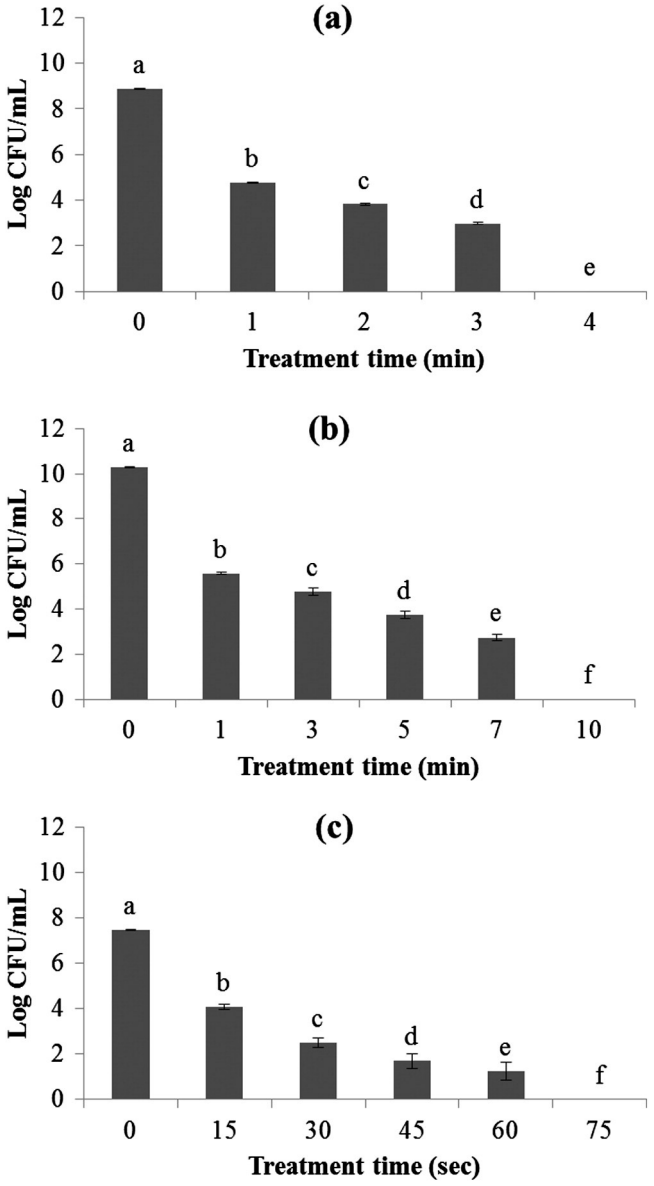

Fig. 2. Inactivation effect of flexible thin-layer DBD plasma against pathogens inoculated onto agar plates. (a) *Escherichia coli* O157:H7, (b) *Listeria monocytogenes*, and (c) *Salmonella* Typhimurium.

reactive species (e.g., O, O_3, H_2O_2, and NO_x) that break covalent bonds and initiate various chemical reactions. However, UV is controversial because some studies reported that UV photons play a negligible role in APP (Deng, Shi, & Kong, 2006; Korachi & Aslan, 2011; Niemira, 2012). Jayasena et al. (2015) showed the emission spectrum of the discharge in thin-layer DBD plasma and observed nitrogen and oxygen molecular spectra because of the ambient air used in the plasma system. Laroussi and Leipold (2004) proposed that air plasmas are excellent source of reactive oxygen species and reactive nitrogen species, such as hydroxyl (OH), ozone (O_3), atomic oxygen (O), NO, and NO_2 and showed that these species are obtained from a DBD operated in atmospheric pressure air. These reactive species have been reported to be the most important agents that participate in pathogen inactivation (Deng et al., 2006; Lee, Paek, Ju, & Lee, 2006; Niemira, 2012). Among the antimicrobial agents generated by APP, ozone has the strong effects on microorganism inactivation (Kim et al., 2011; Lee et al., 2011; Moisan et al., 2002) and can be used alone as a sterilization treatment. When

E. coli K-12 were transformed with pACYC184 plasmid DNA and exposed to short-term ozone treatment, cell viability was not affected. However, prolonged ozone exposure affects DNA and significantly decreases cell viability (Komanapalli & Lau, 1996). In the present study, the ozone concentration generated by flexible thin-layer DBD plasma was found to exceed 200 ppm (data not shown). Leipold et al. (2011) reported that ozone generated by plasma inside packaging has an important role to inactivate *L. monocytogenes*.

Fig. 2 shows that *L. monocytogenes* (a Gram-positive bacterium) inactivation required a longer treatment time compared to that needed to inactivate *E. coli* O157:H7 and *S.* Typhimurium (Gram-negative bacteria). APP treatment were more effective against Gram-negative than Gram-positive bacteria (Dirks et al., 2012; Shi et al., 2011; Korachi, Gurol, & Aslan, 2010; Lee et al., 2006). The relative ineffectiveness of APP against Gram-positive bacteria can be explained by the fact that these cells are surrounded by peptidoglycan structures that resist chemicals (Dirks et al., 2012; Laroussi, 2002; Laroussi, Richardson, & Dobbs, 2002; Montie, Kelly-Wintenberg, & Reece Roth, 2000).

Treatment with flexible thin-layer DBD plasma for 10 min decreased the populations of *E. coli* O157:H7 and *L. monocytogenes* on sliced cheddar cheese by 3.2 and 2.1 Log CFU/g, respectively. In the case of *S.* Typhimurium, the population on sliced cheddar cheese was significantly decreased by plasma treatment and no viable cells were detected after 10 min of treatment (Fi). Song et al. (2009) reported that the D-values of the 3-strain *L. monocytogenes* cocktail, calculated based on the survival curves, were 71.43, 62.50, 19.65, and 17.27 s for sliced cheese exposed to 75-, 100-, 125-, and 150-W APP treatments, respectively. The D-values of the inoculated *E. coli* and *Staphylococcus aureus* were 6.85 and 16.95 min respectively using DBD plasma to treat sliced cheese (Lee et al., 2012b).

However, as shown in Fig. 2 and Fig. 3, flexible thin-layer DBD plasma was less effective on sliced cheddar cheese than on agar plates. The calculated D-values of this plasma against *E. coli* O157:H7, *L. monocytogenes*, and *S.* Typhimurium were 0.70, 1.19, and 0.19 min on the agar plates and 3.10, 4.88, and 1.74 min on the sliced cheddar cheese, respectively. Noriega, Shama, Laca, Díaz, and Kong (2011) reported that APP treatment led to a maximal reduction in *L. innocua* on membrane filters, but was less effective on chicken muscle and skin. Similarly, Song et al. (2009) showed that *L. monocytogenes* inactivation significantly differed when the pathogen was inoculated on ham versus cheese. The authors attributed this difference to the fact that the surface of the sliced ham was rougher than that of the sliced cheese, providing numerous sites for *L. monocytogenes* to attach and potentially escape antimicrobial treatment. The influence of the surface topography on the efficacy of plasma treatment was observed using scanning electron micrographs (Fernández, Noriega, & Thompson, 2013). The micrographs show that surfaces of lettuce, strawberry and potato tissue have stomata, convolutions and some walls which create physical barriers and conceal some *Salmonella* cells. For this reason, longer plasma treatment time is needed to decontaminate the lettuce, strawberry or potato tissue rather than the membrane filter. These results were similar to that of other studies showing that the efficacy of APP is greatly affected by the surface characteristics of the serving sample (Fernandez, Shearer, Wilson, & Thompson, 2012; Lee et al., 2011; Yun et al., 2010).

On the other hand, when sealed-type plasma is switched off after the period of treatment, the remaining reactive species are trapped inside the sealed container during the post-treatment duration and this will be one of the key treatment parameters that determine consistency in bacterial inactivation (Leipold et al., 2011; Rød et al., 2012). Yong et al. (2015) reported that additional 3–4 and 1.5–2 decimal reductions of pathogens were inoculated on agar plates and cheese slices, respectively, for 5 min of post-treatment duration using square-type encapsulated DBD plasma system. Therefore, a further inactivation effect can be expected from the present system used in this study.

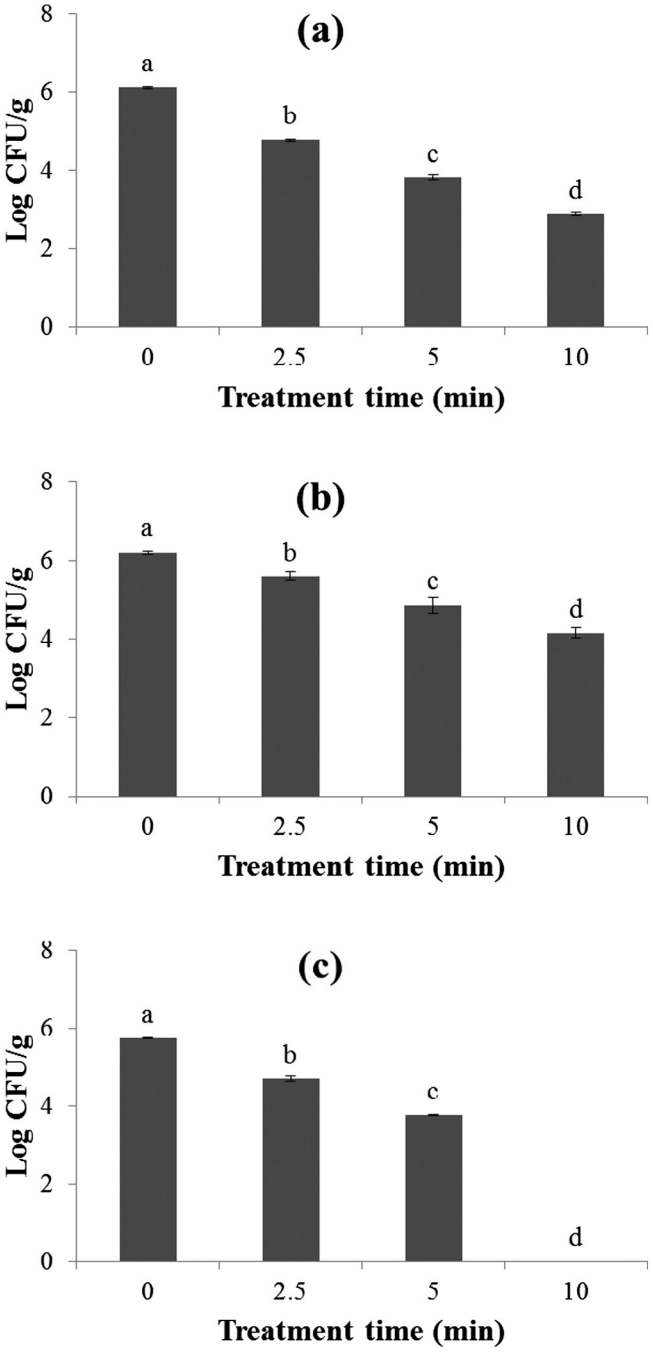

Fig. 3. Inactivation effect of flexible thin-layer DBD plasma against pathogens inoculated onto sliced cheddar cheese. (a) *Escherichia coli* O157:H7, (b) *Listeria monocytogenes*, and (c) *Salmonella* Typhimurium.

3.2. pH

When flexible thin-layer DBD plasma was applied to sliced cheddar cheese for more than 5 min, pH values declined significantly (Table 1). Indirect and DBD plasma-treated pork loins were recently shown to have significantly lower pH values than untreated pork loins (Fröhling et al., 2012; Kim et al., 2013). With extended non-thermal APP treatments, pH values were also found to decrease in distilled water,

Table 1
pH changes in sliced cheddar cheese treated with flexible thin-layer DBD plasma.

Treatment time (min)	pH
0	$6.42^{a} \pm 0.017$*
2.5	$6.39^{ab} \pm 0.010$
5	$6.33^{b} \pm 0.035$
10	$6.29^{c} \pm 0.021$

[a–c]Different letters within same column differ significantly ($p < 0.05$).
* Mean ± standard deviation.

orange juice, and algal cultures (Korachi & Aslan, 2011; Shi et al., 2011; Tang, Lu, Laroussi, & Dobbs, 2008). Tang et al. (2008) suggested that the following acid-forming reactions explain the plasma-induced decrease in pH:

$$2NO + H_2O + O_3 \rightarrow 2HNO_3 \quad (1)$$

$$NO + O_3 \rightarrow NO_2 + O_2 \quad (2)$$

$$NO_2 + OH\bullet \rightarrow HNO_3. \quad (3)$$

This hypothesis was supported by Fröhling et al. (2012) who reported that higher amounts of acidogenic molecules, such as NO_x, form as a result of indirect plasma treatment and accumulate on the surface of meat samples. Korachi and Aslan (2011) suggested that the increase in H^+, which is dissociated from bacterial molecules or H_2O, also contributes to the reduction in pH observed following plasma treatment. pH fluctuation is known as a stress factor in bacteria and can cause cell death. However, we observed a slight decrease in pH—not extreme acidity or alkalinity—in response to plasma treatment. Consistent with our findings, Korachi et al. (2010) and Shi et al. (2011) showed that the pH decrease in orange juice and distilled water induced by non-thermal APP plays an insignificant role in pathogen inactivation.

3.3. TBARS values

TBARS values were calculated to determine the effect of flexible thin-layer DBD plasma on lipid oxidation in sliced cheddar cheese (Table 2). In response to 5- and 10-min plasma treatments, the TBARS values of sliced cheddar cheese increased significantly compared to those of the non-treated samples. The increase in TBARS value of pork butt and beef loin with thin-layer DBD plasma treatment was also observed in a previous study (Jayasena et al., 2015). Kim et al. (2013) reported that the TBARS values of DBD plasma-treated pork loins were greater than that of the control. Moreover, higher TBARS values were obtained when a combination of He and O_2 was used as a carrier gas than when He was used alone. Treatment with APP also increases the TBARS values of bresaola packaged inside polyethylene bags, with increasing power, treatment time, and storage time (Rød et al., 2012). When cheddar cheese is treated by low-dose gamma irradiation, radical species induce lipid oxidation (Seisa et al., 2004). Similar to irradiation, plasma can generate radicals and compromise the functions of fatty acids (especially unsaturated fatty acids), thereby inducing lipid oxidation (Kim et al., 2013; Laroussi, 1996; Montie et al., 2000). Ozone generated by flexible thin-layer DBD plasma can also react with fatty acids, inducing the production of malonaldehyde, a primary product of hydroperoxide homolytic cleavage during oxidation (Goldstein, Lodi, Collinson, & Balchum, 1969; Roehm, Hadley, & Menzel, 1971). However, lipid oxidation is not observed in APP-treated bacon, and cooked egg white, and yolk (Kim et al., 2011; Lee et al., 2012a). Therefore, the changes in TBARS values might depend on the plasma type, carrier gas or characteristics of the plasma-treated sample, such as its fat content or fatty acid composition (Kim et al., 2011).

Table 2
TBARS values in sliced cheddar cheese treated with flexible thin-layer DBD plasma.

Treatment time (min)	TBARS value (mg malondialdehyde/kg)
0	$0.132^{c} \pm 0.021$*
2.5	$0.141^{bc} \pm 0.022$
5	$0.161^{ab} \pm 0.006$
10	$0.183^{a} \pm 0.027$

[a–c]Different letters within same column differ significantly ($p < 0.05$).
* Mean ± standard deviation.

Table 3
Surface color values of sliced cheddar cheese treated by flexible thin-layer DBD plasma.

Treatment time (min)	L*	a*	b*	Total color difference (ΔE)
0	$74.91^{a} \pm 0.234$*	18.69 ± 0.107	$38.10^{b} \pm 0.367$	86.10 ± 0.325
2.5	$74.84^{a} \pm 0.291$	18.46 ± 0.274	$38.01^{b} \pm 0.367$	85.95 ± 0.478
5	$74.21^{b} \pm 0.106$	19.35 ± 0.132	$38.43^{ab} \pm 0.146$	85.78 ± 0.334
10	$74.01^{b} \pm 0.283$	19.36 ± 0.183	$38.99^{a} \pm 0.497$	85.87 ± 0.085

[a,b]Different letters within same column differ significantly ($p < 0.05$).
* Mean ± standard deviation.

3.4. Surface color

The lightness (L*), redness (a*), and yellowness (b*) values and total color difference (ΔE) were calculated from the surface color measurements of flexible thin-layer DBD plasma-treated sliced cheddar cheese as given in Table 3. The sliced cheddar cheese used in the present study contained oleoresin paprika for coloring. Radicals and oxidizing species are known to significantly reduce the yellow pigments of paprika (e.g., zeaxanthin, capsolutein, violaxanthin, β-carotene, and β-cryptoxanthin) (Bors, Saran, & Michel, 1982; Krinsky & Yeum, 2003; Topuz & Ozdemir, 2003). However, we observed a significant decrease in L*-value and increase in a*-value for sliced cheddar cheese treated with flexible thin-layer DBD plasma and no differences in b*-value and ΔE (Table 3). Lee et al. (2012b) observed a decrease in L*-values but an increase in a*-values of DBD-treated cheese slices, suggesting a more brown color. Food browning is typically the consequence of reactions between amino groups (proteins, peptides, amino acids, and amines) with reducing sugars, oxidized lipids, vitamin C, and quinones. Reactions involving quinones are known as enzymatic browning reactions, whereas those involving other groups are considered nonenzymatic browning reactions (Zamora & Hidalgo, 2005). Konteles, Sinanoglou, Batrinou, and Sflomos (2009) proposed that the Maillard reaction, a nonenzymatic browning reaction, might occur between amino acids or milk proteins and milk lactose. Lipid oxidation products might also produce brown-colored oxypolymers from milk proteins. However, we are unable to conclude that

Table 4
Sensory evaluation of sliced cheddar cheese treated by flexible thin-layer DBD plasma.

Sensory parameter	Treatment time (min)			
	0	2.5	5	10
Appearance	4.97 ± 0.249*	5.10 ± 0.337	5.00 ± 0.486	4.83 ± 0.501
Color	4.88 ± 0.270	5.00 ± 0.373	4.90 ± 0.428	5.10 ± 0.412
Flavor	$5.18^{a} \pm 0.169$	$5.08^{ab} \pm 0.409$	$4.80^{ab} \pm 0.422$	$4.78^{b} \pm 0.533$
Taste	$5.18^{a} \pm 0.206$	$4.90^{ab} \pm 0.34$	$4.48^{b} \pm 0.617$	$4.65^{b} \pm 0.580$
Off-odor	$4.97^{c} \pm 0.105$	$5.23^{bc} \pm 0.376$	$5.53^{ab} \pm 0.472$	$5.73^{a} \pm 0.558$
Overall acceptance	$5.23^{a} \pm 0.249$	$5.08^{a} \pm 0.369$	$4.68^{b} \pm 0.553$	$4.60^{b} \pm 0.543$

[a–c]Different letters within same row differ significantly ($p < 0.05$).
* Mean ± standard deviation.

browning reactions are solely responsible for color changes in cheddar cheese due to its complexity.

3.5. Sensory evaluation

When flexible thin-layer DBD plasma was applied to sliced cheddar cheese for 10 min, significant reductions in flavor were observed, while taste, off-odor, and overall acceptance change after 5 and 10 min of treatment compared to the control (Table 4). Similarly, Lee et al. (2012b) observed significant reductions in the flavor, odor, and overall acceptability of DBD plasma-treated cheese slices. The lipid oxidation occurring in sliced cheddar cheese treated by flexible thin-layer DBD plasma, may possibly affect the sensory characteristics. Free radicals, which are precursors of lipid hydroperoxides, can cause lipid oxidation and lead to the production of secondary oxidation products such as alkanes, alkenes, aldehydes, alcohols, ketones, and acids (Konteles et al., 2009; Nawar, 1985). These lipid oxidation byproducts produce off-odors described as metallic, fishy, rancid, and oxidized (Kim et al., 2011; Kochhar, 1996). Lee et al. (2012a) showed that plasma treatment caused insignificant sensory changes in cooked egg whites, whereas significant sensory reductions were found in treated cooked egg yolks. Sensory changes in plasma-treated foods possibly vary depending on the characteristics of the food such as its fatty acid composition, fat content, and protein content. Therefore, it is important to determine the factors affecting sensory changes in plasma-treated foods in order to prevent any negative effects of plasma treatment.

4. Conclusion

It can be concluded based on the results of the present study that flexible thin-layer DBD plasma is a suitable method for pathogen inactivation on sliced cheddar cheese. However, the application of this technique is limited due to its effects on sensory quality. Therefore, further studies are needed to optimize the system, especially the sensory quality aspects, for successful industrial application.

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References

Bors, W., Saran, M., & Michel, C. (1982). Radical intermediates involved in the bleaching of the carotenoid crocin. Hydroxyl radicals, superoxide anions and hydrated electrons. *International Journal of Radiation Biology*, *41*, 493–501.

Deng, X., Shi, J., & Kong, M.G. (2006). Physical mechanisms of inactivation of *Bacillus subtilis* spores using cold atmospheric plasmas. *IEEE Transactions on Plasma Science*, *34*, 1310–1316.

Dirks, B.P., Dobrynin, D., Fridman, G., Mukhin, Y., Fridman, A., & Quinlan, J.J. (2012). Treatment of raw poultry with nonthermal dielectric barrier discharge plasma to reduce *Campylobacter jejuni* and *Salmonella enterica*. *Journal of Food Protection*, *75*, 22–28.

Fernández, A., Noriega, E., & Thompson, A. (2013). Inactivation of *Salmonella enterica* serovar Typhimurium on fresh produce by cold atmospheric gas plasma technology. *Food Microbiology*, *33*, 24–29.

Fernandez, A., Shearer, N., Wilson, D.R., & Thompson, A. (2012). Effect of microbial loading on the efficiency of cold atmospheric gas plasma inactivation of *Salmonella enterica* serovar Typhimurium. *International Journal of Food Microbiology*, *152*, 175–180.

Fröhling, A., Durek, J., Schnabel, U., Ehlbeck, J., Bolling, J., & Schlüter, O. (2012). Indirect plasma treatment of fresh pork: Decontamination efficiency and effects on quality attributes. *Innovative Food Science & Emerging Technologies*, *16*, 381–390.

Goldstein, B.D., Lodi, C., Collinson, C., & Balchum, O.J. (1969). Ozone and lipid peroxidation. *Archives of Environmental Health*, *18*, 631–635.

Haas, J., Behsnilian, D., & Schubert, H. (1996). Determination of the heat resistance of bacterial spores by the capillary tube method. I. Calculation of two borderline cases describing quasi-isothermal conditions. *Lebensmittel-Wissenschaft und Technologie*, *29*, 197–202.

Jayasena, D.D., Kim, H.J., Yong, H.I., Park, S., Kim, K., Choe, W., et al. (2015). Flexible thin-layer dielectric barrier discharge plasma treatment of pork butt and beef loin: Effects on pathogen inactivation and meat-quality attributes. *Food Microbiology*, *46*, 51–57.

Jung, Y. K., Jeon, H. J., Jung, S., Choe, J. H., Lee, J. H., Heo, K. N., et al. (2011). Comparison of quality traits of thigh meat from Korean native chickens and broilers. *Korean Journal for Food Science of Animal Resources*, *31*, 684–692.

Kim, H.J., Yong, H.I., Park, S., Choe, W., & Jo, C. (2013). Effects of dielectric barrier discharge plasma on pathogen inactivation and the physicochemical and sensory characteristics of pork loin. *Current Applied Physics*, *13*, 1420–1425.

Kim, B., Yun, H., Jung, S., Jung, Y., Jung, H., Choe, W., et al. (2011). Effect of atmospheric pressure plasma on inactivation of pathogens inoculated onto bacon using two different gas compositions. *Food Microbiology*, *28*, 9–13.

Koch, J., Dworak, R., Prager, R., Becker, B., Brockmann, S., Wicke, A., et al. (2010). Large listeriosis outbreak linked to cheese made from pasteurized milk, Germany, 2006–2007. *Foodborne Pathogens and Disease*, *7*, 1581–1584.

Kochhar, S.P. (1996). Oxidation pathways to the formation of off-flavours. In M.J. Saxby (Ed.), *Food taints and off-flavours* (pp. 168–225) (2nd eds.). London: Blackie Academic & Professional.

Komanapalli, I.R., & Lau, B.H.S. (1996). Ozone-induced damage of *Escherichia coli* K-12. *Applied Microbiology and Biotechnology*, *46*, 610–614.

Konteles, S., Sinanoglou, V.J., Batrinou, A., & Sflomos, K. (2009). Effects of γ-irradiation on *Listeria monocytogenes* population, colour, texture and sensory properties of Feta cheese during cold storage. *Food Microbiology*, *26*, 157–165.

Korachi, M., & Aslan, N. (2011). The effect of atmospheric pressure plasma corona discharge on pH, lipid content and DNA of bacterial cells. *Plasma Science and Technology*, *13*, 99.

Korachi, M., Gurol, C., & Aslan, N. (2010). Atmospheric plasma discharge sterilization effects on whole cell fatty acid profiles of *Escherichia coli* and *Staphylococcus aureus*. *Journal of Electrostatics*, *68*, 508–512.

Krinsky, N.I., & Yeum, K.J. (2003). Carotenoid–radical interactions. *Biochemical and Biophysical Research Communications*, *305*, 754–760.

Laroussi, M. (1996). Sterilization of contaminated matter with an atmospheric pressure plasma. *IEEE Transactions on Plasma Science*, *24*, 1188–1191.

Laroussi, M. (2002). Nonthermal decontamination of biological media by atmospheric-pressure plasmas: Review, analysis, and prospects. *IEEE Transactions on Plasma Science*, *30*, 1409–1415.

Laroussi, M. (2005). Low temperature plasma-based sterilization: Overview and state-of-the-art. *Plasma Processes and Polymers*, *2*, 391–400.

Laroussi, M., & Leipold, F. (2004). Evaluation of the roles of reactive species, heat, and UV radiation in the inactivation of bacterial cells by air plasmas at atmospheric pressure. *International Journal of Mass Spectrometry*, *23*, 81–86.

Laroussi, M., Richardson, J.P., & Dobbs, F.C. (2002). Effects of nonequilibrium atmospheric pressure plasmas on the heterotrophic pathways of bacteria and on their cell morphology. *Applied Physics Letters*, *81*, 772–774.

Lee, H.J., Jung, H., Choe, W., Ham, J.S., Lee, J.H., & Jo, C. (2011). Inactivation of *Listeria monocytogenes* on agar and processed meat surfaces by atmospheric pressure plasma jets. *Food Microbiology*, *28*, 1468–1471.

Lee, H.J., Jung, S., Jung, H., Park, S., Choe, W., Ham, J.S., et al. (2012a). Evaluation of dielectric barrier discharge plasma system for inactivating pathogens on cheese slices. *Journal of Animal Science and Technology*, *54*, 191–198.

Lee, K., Paek, K.H., Ju, W., & Lee, Y. (2006). Sterilization of bacteria, yeast, and bacterial endospores by atmospheric-pressure cold plasma using helium and oxygen. *The Journal of Microbiology*, *44*, 269.

Lee, H.J., Song, H.P., Jung, H.S., Choe, W., Ham, J.S., Lee, J.H., et al. (2012b). Effect of atmospheric pressure plasma jet on inactivation of *Listeria monocytogenes*, quality, and genotoxicity of cooked egg white and yolk. *Korean Journal for Food Science of Animal Resources*, *32*, 561–570.

Leipold, F., Schultz-Jensen, N., Kusano, Y., Bindslev, H., & Jacobsen, T. (2011). Decontamination of objects in a sealed container by means of atmospheric pressure plasmas. *Food Control*, *22*, 1296–1301.

Makino, S.I., Kawamoto, K., Takeshi, K., Okada, Y., Yamasaki, M., Yamamoto, S., et al. (2005). An outbreak of food-borne listeriosis due to cheese in Japan, during 2001. *International Journal of Food Microbiology*, *104*, 189–196.

McCollum, J.T., Williams, N.J., Beam, S.W., Cosgrove, S., Ettestad, P.J., Ghosh, T.S., et al. (2012). Multistate outbreak of *Escherichia coli* O157:H7 infections associated with in-store sampling of an aged raw-milk Gouda cheese, 2010. *Journal of Food Protection*, *75*, 1759–1765.

Moisan, M., Barbeau, J., Crevier, M.C., Pelletier, J., Philip, N., & Saoudi, B. (2002). Plasma sterilization. Methods and mechanisms. *Pure and Applied Chemistry*, *74*, 349–358.

Montie, T.C., Kelly-Wintenberg, K., & Reece Roth, J. (2000). An overview of research using the one atmosphere uniform glow discharge plasma (OAUGDP) for sterilization of surfaces and materials. *IEEE Transactions on Plasma Science*, *28*, 41–50.

Nawar, W.W. (1985). Lipids. In O.R. Fennema (Ed.), *Food chemistry (2nd eds.). Revised and expanded.* (pp. 139–244). New York: Marcel Dekke.

Niemira, B.A. (2012). Cold plasma reduction of *Salmonella* and *Escherichia coli* O157:H7 on almonds using ambient pressure gases. *Journal of Food Science*, *77*, M171–M175.

Noriega, E., Shama, G., Laca, A., Díaz, M., & Kong, M.G. (2011). Cold atmospheric gas plasma disinfection of chicken meat and chicken skin contaminated with *Listeria innocua*. *Food Microbiology*, *28*, 1293–1300.

Rød, S.K., Hansen, F., Leipold, F., & Knøchel, S. (2012). Cold atmospheric pressure plasma treatment of ready-to-eat meat: Inactivation of *Listeria innocua* and changes in product quality. *Food Microbiology*, *30*, 233–238.

Roehm, J.N., Hadley, J.G., & Menzel, D.B. (1971). Oxidation of unsaturated fatty acids by ozone and nitrogen dioxide. *Archives of Environmental Health*, *23*, 142–148.

Schoder, D., Stessl, B., Szakmary-Brändle, K., Rossmanith, P., & Wagner, M. (2014). Population diversity of *Listeria monocytogenes* in quargel (acid curd cheese) lots recalled during the multinational listeriosis outbreak 2009/2010. *Food Microbiology*, *39*, 68–73.

Seisa, D., Osthoff, G., Hugo, C., Hugo, A., Bothma, C., & Van der Merwe, J. (2004). The effect of low-dose gamma irradiation and temperature on the microbiological and chemical changes during ripening of cheddar cheese. *Radiation Physics and Chemistry*, *69*, 419–431.

Shi, X.M., Zhang, G.J., Wu, X.L., Li, Y.X., Ma, Y., & Shao, X.J. (2011). Effect of low-temperature plasma on microorganism inactivation and quality of freshly squeezed orange juice. *IEEE Transactions on Plasma Science*, *39*, 1591–1597.

Song, H.P., Kim, B., Choe, J.H., Jung, S., Moon, S.Y., Choe, W., et al. (2009). Evaluation of atmospheric pressure plasma to improve the safety of sliced cheese and ham inoculated by 3-strain cocktail *Listeria monocytogenes*. *Food Microbiology*, *26*, 432–436.

Song, Y., Liu, D., Ji, L., Wang, W., Zhao, P., Quan, C., et al. (2012). The inactivation of resistant *Candida albicans* in a sealed package by cold atmospheric pressure plasmas. *Plasma Processes and Polymers*, *9*, 17–21.

Tang, Y.Z., Lu, X.P., Laroussi, M., & Dobbs, F.C. (2008). Sublethal and killing effects of atmospheric-pressure, nonthermal plasma on eukaryotic microalgae in aqueous media. *Plasma Processes and Polymers*, *5*, 552–558.

Topuz, A., & Ozdemir, F. (2003). Influences of γ-irradiation and storage on the carotenoids of sun-dried and dehydrated paprika. *Journal of Agricultural and Food Chemistry*, *51*, 4972–4977.

Torres-Vitela, M.R., Mendoza-Bernardo, M., Castro-Rosas, J., Gomez-Aldapa, C.A., Garay-Martinez, L.E., Navarro-Hidalgo, V., et al. (2012). Incidence of *Salmonella*, *Listeria monocytogenes*, *Escherichia coli* O157:H7, and staphylococcal enterotoxin in two types of Mexican fresh cheeses. *Journal of Food Protection*, *75*, 79–84.

Yong, H.I., Kim, H.J., Park, S., Alahakoon, A.U., Kim, K., Choe, W., et al. (2015). Evaluation of pathogen inactivation on sliced cheese induced by encapsulated atmospheric pressure dielectric barrier discharge plasma. *Food Microbiology*, *46*, 46–50.

Yun, H., Kim, B., Jung, S., Kruk, Z.A., Kim, D.B., Choe, W., et al. (2010). Inactivation of *Listeria monocytogenes* inoculated on disposable plastic tray, aluminum foil, and paper cup by atmospheric pressure plasma. *Food Control*, *21*, 1182–1186.

Zamora, R., & Hidalgo, F.J. (2005). Coordinate contribution of lipid oxidation and Maillard reaction to the nonenzymatic food browning. *Critical Reviews in Food Science and Nutrition*, *45*, 49–59.