

Evaluation of the microbiological safety, quality changes, and genotoxicity of chicken breast treated with flexible thin-layer dielectric barrier discharge plasma

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Abstract The microbiological safety, quality changes, and genotoxicity of chicken breasts treated with flexible thin-layer dielectric barrier discharge (FTDBD) plasma inside a sealed package were investigated. Following 10 min plasma treatment, the numbers of total aerobic bacteria, *Listeria monocytogenes*, *Escherichia coli*, and *Salmonella* Typhimurium were reduced by 3.36, 2.14, 2.73, and 2.71 Log CFU/g, respectively. Color L* and b* values increased whereas a* value decreased following plasma treatment with increasing exposure duration. Lipid oxidation was unaffected by plasma treatment. In sensory evaluation, flavor decreased and off-flavor increased with extended plasma exposure time, however, these changes had no effect on acceptability. Increased cohesiveness was the only texture profile analysis parameter that changed following plasma treatment. No genotoxicity was detected in plasma-treated chicken breast using the *Salmonella* mutagenicity assay. Therefore, FTDBD plasma is applicable since it is able to improve microbiological safety with minimal changes in sensory properties of the chicken breast.

Keywords: chicken breast, plasma, quality, safety, genotoxicity

Introduction

The number of pathogens able to cause food-borne illness is estimated at 31 (1). These food-borne pathogens are often found in retail raw meats; however, the type and amount of existent pathogens differs depending on the type of meat (2). Chicken meat, in particular, is highly susceptible to cross-contamination by enteric pathogens that can cause food-borne illness, such as *Salmonella* and *Listeria*, during slaughter (3). Zhao *et al.* (2) reported that 38.7 and 4.2% of 212 retail chicken samples were contaminated by *Escherichia coli* and *Salmonella*, respectively. In the case of *Listeria*, the prevalence was 26% (23 of 89 chicken meat samples) (4). Therefore, inactivation of pathogens present in chicken meat is essential for safe consumption.

Plasma, which is an ionized gas or the fourth state of matter, is generated by the interaction between electromagnetic fields and gas, especially, when the field is sufficiently strong (5). Plasma technology has been studied as a non-thermal sterilization method for improving food safety. The effect of this technology is significant

since, unlike thermal sterilization, it does not cause nutritional and quality changes (6,7); in addition, it is more cost-effective and easy to install compared with other non-thermal sterilization technologies such as gamma irradiation, electron-beam, and high pressure (8,9). Inactivation of pathogens during plasma discharge is achieved by several factors including the electric field, UV photons, charged particles, and reactive species such as O, O₃, OH, H₂O₂, NO, or NO₂ (10-12). A number of studies have established the antimicrobial effect of plasma on meat and meat products (9,13,14).

Secondary contamination due to processing conditions (staff or equipment) is an unavoidable problem in slaughterhouses (15). Therefore, sanitation following packaging is required in order to prevent secondary contamination (16) and quality deterioration; this is thought to be one of the greatest advantages of irradiation or high-pressure treatment. For this reason, researchers have attempted to develop a type of plasma sealed in a bottle (17), container (16,18), or package (6,19). A recent study by Jayasena *et al.* (6) demonstrated the effect of a sealed package with flexible thin-layer dielectric

barrier discharge (FTDBD) plasma on pork butt and beef loin, thus establishing an option for using the FTDBD plasma system.

Bacterial inactivation effect of plasma has been tested on chicken meat but following quality changes has not been investigated yet (3,9,13). Therefore, the aim of this study was to evaluate the inactivation effect of FTDBD plasma on chicken breasts, generated within a sealed package, and observe changes in quality and the genotoxicological safety using the *Salmonella* mutagenicity assay following different durations of plasma exposure.

Materials and Methods

Sample preparation and sterilization Raw chicken breasts were purchased from a local market in Seoul, Korea and divided into two sections. One section was cut to the same size (25×25×7 mm) and vacuum-packaged. Then, the chicken breast samples were sterilized for inoculation testing with irradiation (35 kGy) using a linear electron-beam RF accelerator (10 MeV, 40 kW; EB Tech, Daejeon, Korea). The other section of chicken breast was treated with DBD plasma directly without sterilization step and used to determine the number of total aerobic bacteria, physicochemical properties and toxicological safety.

Treatment with FTDBD plasma A flexible package system which is designed for generating DBD plasma within the package was prepared using the conductive layer of a commercial, zippered food package (129×199 mm) as the powered electrode (6). While the inner patterned electrode was grounded, a bipolar square-waveform voltage of 15 kHz was applied to the outer electrode. Plasma was generated at the surface of the inner electrode at 100 W peak power and 2 W average power. As an operating gas, the atmospheric air was used.

After the DBD plasma treatment, the respective samples were used for microbial analysis and instrumental color measurement immediately. The other samples were stored under at 4°C until the following day and analysis of other physicochemical properties, sensory parameters, and toxicological safety.

Microbial analysis In order to analysis the total aerobic bacteria, chicken sample (5 g) was mixed with 45 mL of sterile saline solution (0.85%) using a stomacher BagMixer[®] 400 (Interscience Co., Saint Nom, France). Then, the samples were decimally diluted using sterile saline solution. Total plate count agar (Difco Laboratories, Franklin Lakes, NJ, USA) was prepared for counting the total number of aerobic microbes. The plates were incubated at 37°C for 48 h. All colonies were counted and the number of microorganisms was expressed as Log CFU/g.

Inoculation test *Escherichia coli* O157:H7 (KCCM 40406), *Salmonella* Typhimurium (KCTC 1925), and *Listeria monocytogenes* (KCTC 3569)

were cultivated in tryptic soy broth (TSB), nutrient broth, and TSB containing 0.6% yeast extract, respectively (Difco Laboratories), at 37°C for 48 h. Then, the cultures were centrifuged (2,265×g for 15 min) in a refrigerated centrifuge (UNION 32R; Hanil Science Industrial Co. Ltd., Incheon, Korea). The obtained pellets were washed twice with sterile saline solution (0.85%) and suspended in sterile saline solution to achieve a viable cell density of approximately 10⁸-10⁹ CFU/mL. The irradiation-sterilized chicken breast samples were removed from the packages and inoculated with the 100 µL of test-culture suspensions. Subsequent to spreading the suspensions on the chicken breasts, the meat samples were kept under sterile conditions at room temperature for 10 min to enable attachment of the microorganisms to the samples.

After plasma treatment, inoculated samples were blended with 45 mL of sterile saline as described above. Decimal dilutions were prepared with the sterile saline solution. The media used for the recording the growth of *E. coli* O157:H7, *L. monocytogenes*, and *S. Typhimurium* were tryptic soy agar (TSA; Difco Laboratories), TSA containing 0.6% yeast extract (Difco Laboratories), and nutrient agar (Difco Laboratories), respectively. Incubation of plates and colony counting were done as explained above.

Instrumental color measurement The color of plasma-treated and untreated chicken breast samples were measured by a colorimeter (Spectrophotometer, CM-3500d; Konica Minolta Sensing, Inc., Osaka, Japan) using CIE L^{*}-, a^{*}, and b^{*}-value. The instrument was calibrated with a standard white and black plate before analysis. The color values were monitored using a computerized system controlled by Spectra Magic software (Konica Minolta Sensing, Inc.).

Measurement of 2-thiobarbituric acid reactive substances (TBARS) Each chicken breast sample (3 g) was homogenized (Ika Laboratory Equipment, Staufen im Breisgau, Germany) with 9 mL of distilled water and 50 µL of 7.2% butylated hydroxytoluene (in ethanol). Then the homogenate (1 mL) was transferred to a 15-mL test tube and mixed with 2 mL of a thiobarbituric acid (20 mmol/L)/trichloroacetic acid (15%) solution. Next, the test tubes were heated in a 90°C water bath for 30 min, cooled in cold water, and centrifuged (Hanil Science Industrial Co. Ltd.) at 2,090×g for 10 min. A spectrophotometer (DU 530; Beckman Instruments Inc., Brea, CA, USA) was used to measure the absorbance of the supernatant at 532 nm. The TBARS value (mg malondialdehyde/kg sample) was calculated using a standard curve.

Texture analysis Plasma treated and untreated chicken breasts were minced and separately prepared (4 cm in diameter x2 cm thickness; approximately 20 g), then cooked to reach an internal temperature of 75°C. Texture of sample was measured by a texture analyzer (TA-XT Plus; Stable Micro Systems Ltd., Surrey, UK) by compressing the centers of the cooked meat samples. A probe (75 mm in diameter) was compressed twice to 75% of their original height at a test speed of 2.00 mm/s and a trigger force of 50 g. The

Exponent Lite Texture Analysis software (Stable Micro System Ltd.) was used for texture analysis; hardness, adhesiveness, springiness, cohesiveness, gumminess, and chewiness were recorded. In each treatment combination, three replicate samples were measured.

Sensory evaluation FTDBD plasma-treated and untreated chicken breasts were cut into sections (25×25×7 mm) and cooked on an electric grill featuring double heating surfaces (1,400 W, NovaEMG-533; Evergreen Enterprise, Yongin, Korea) until the internal temperature of sample reached 72°C. The temperature of the center of meat sample was monitored by a digital thermometer (YF-160A, Type-K; YFE, Hsinchu, Taiwan). For evaluation, the samples were transferred in randomly coded white dishes and served together with drinking water. Eight semi-trained panelists who have had at least 1 year of experience in sensory analysis of meat treated with non-thermal processing, evaluated the cooked samples for color, appearance, taste, off-flavor, and overall acceptability by a 9-point hedonic scale (from extreme dislike=1 to extreme like=9). The sensory evaluation was run three times independently for replicated study.

Mutagenicity assay The mutagenicity assay was conducted with ethanolic extracts of plasma-treated sample for 0 and 10 min. A total of 100 g of each extract was transferred into 900 mL of 70% (v/v) ethanol. The extracts incubated for 8 h at room temperature were filtered by Whatman filter paper No. 4 (Whatman International Ltd., Kent, England). Then, 70% ethanol (900 mL) was added again and the procedure was repeated. Ethanol was removed from the samples using a rotary vacuum evaporator (Rotary Vacuum Evaporator N-11 Eyela; Tokyo Rikakikai Co. Ltd., Tokyo, Japan). The extracts were dried (Freeze dry system, FreeZone 18; Labconco Corp., Kansas City, MO, USA,) following freezing and kept at -70°C before use.

The *Salmonella* mutagenicity assay (Ames test) was conducted according to the methods of Ames *et al.* (20) and Maron and Ames (21). *S. Typhimurium* strains TA98 and TA100 were provided by the Korea Institute of Toxicology (KIT, Daejeon, Korea). The strains were purchased from Molecular Toxicology Inc. originally (Boone, NC, USA) and cultured by the KIT in the Korea Research Institute of Chemical Technology (KRICT, Daejeon, USA). Each strain was first tested for its genetic traits including histidine requirement, deep rough (*rfa*) characteristic, UV sensitivity (*uvrB* mutation), and ampicillin or tetracycline-resistance by R-factor prior to use. The strains were inoculated on nutrient broth No. 2 (Oxoid Co., Ltd., Hampshire, England) and cultured at 37°C for 10 h at 200 rpm (Vision Scientific Co., Incheon, Korea) to a cell density of 2×10^9 CFU/mL. The tested sample doses were 1,250, 2,500, and 5,000 µg sample per plate.

S9 mix was obtained from the Oriental Yeast Co. Ltd. (Lot No. 0042101; Oriental Yeast Co., Ltd., Tokyo, Japan); 5% S9 mix was prepared using the S9 mix fraction and a cofactor (Lot No. 999902; Wako Co., Tokyo, Japan). Treatment concentration was 500 µL/plate. The positive controls included 4-nitroquinoline-1-oxide (4-NQO,

Sigma-Aldrich, St. Louis, MO, USA), 2-Aminoanthracene (2-AA, Sigma-Aldrich) dissolved in deionized distilled water (DDW) or dimethylsulfoxide (DMSO, Sigma-Aldrich), sodium azide (SA, Sigma-Aldrich). Two plates per concentration were used for the direct plate incorporation method; 100 µL of sample combined with 100 µL of bacterial culture (2×10^9 CFU/mL) and 500 µL of the S9 mixture were added to 2 mL of warm top agar (approximately 45°C) containing 0.5 mM histidine-biotin. This mixture was poured onto a minimal glucose agar plate and allowed to solidify. The plates were incubated for 48 h at 37°C and then the number of revertant colonies was enumerated. The negative control was 100 µL of ethanol. The positive control was 4-NQO and SA (100 µL each) when metabolic activation was not incorporated and 2-AA (100 µL) when metabolic activation was incorporated. Mutagenicity was determined using the method of Maron and Ames (21) stating that if the number of revertant colonies in a sample is greater than double that in the negative control and it demonstrates a dose-dependent response, it means statistically significant and considered as positive for mutagenicity.

Statistical analysis Each set of data represents the mean of three replications. One-way analysis of variance was performed with a completely randomized design using the procedure of General Linear Model. Significant differences among mean values were determined using Duncan's multiple comparison test in SAS Release 9.2. (SAS Institute Inc., Cary, NC, USA) with the confidence level of $p < 0.05$. Mean values and standard errors of the mean are reported. All experimental procedures were conducted in triplicate with two observation numbers except for sensory analysis.

Results and Discussion

Inactivation of foodborne pathogens The antibacterial effect of plasma treatment on chicken breasts has been previously demonstrated (3,9,13), although these studies used different types of plasma systems. Jayasena *et al.* (6) inoculated pork butt and beef loin with *L. monocytogenes*, *E. coli* O157:H7, and *S. Typhimurium* and treated the meat for 10 min with FTDBD plasma. The number of these pathogens were reduced by 2.04, 2.54, and 2.68 Log CFU/g, respectively, in pork butt samples and by 1.98, 2.57, and 2.58 Log CFU/g, respectively, in beef loin following plasma treatment for 10 min. The pathogen reduction rate was higher (3.20, 2.10, and 5.80 Log CFU/g, respectively) in sliced cheddar cheese (22). Similar to these previous studies, the results of the current study demonstrate that the number of total aerobic bacteria (Fig. 1) and inoculated pathogens (Table 1) in the chicken breast samples was clearly reduced by FTDBD plasma. No viable aerobic bacterial cells were detected following 10 min plasma treatment (Fig. 1). As shown in Table 1, the populations of the three pathogens in the chicken breast samples decreased with increasing treatment duration ($p < 0.05$); the log-reduction of *L. monocytogenes*, *E. coli* O157:H7, and *S. Typhimurium*

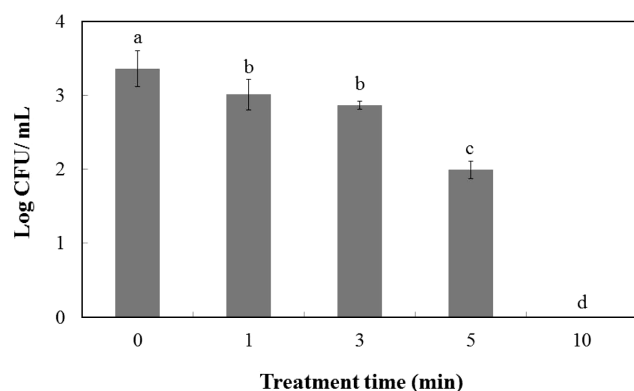


Fig. 1. Effect of flexible thin-layer dielectric barrier discharge plasma on inactivation of total aerobic bacteria in chicken breasts. ^{a-d}Different letters among the treatments differ significantly ($p < 0.05$).

was 2.14, 2.73, and 2.71 Log CFU/g, respectively, quite similar to the values obtained for other previously tested foods (6,9).

Several plasma agents are generated when plasma is discharged that contribute to the inactivation of microorganisms, including electric field, UV photons, charged particles, and reactive species. However, electric field and UV are not major factors in plasma sterilization. Ma *et al.* (23) investigated the effect of electric field on *Staphylococcus aureus* and *E. coli* using a stepwise 60 s application of voltage (0-12 kV) until critical voltage was reached (critical voltage; if voltage exceeds the critical point, the influence of the electric field significantly decreased) (24); however, no significant differences were detected in the inactivation results. UV irradiation is known to trigger the formation of thymine dimers in nucleic acids and can inhibit microorganism growth (11). According to Guo *et al.* (11), the bactericidal effect of UV generated by plasma alters depending on gas and discharge type. UV has a significant effect only when argon or an oxygen/nitrogen mixture gas is used as the operating gas or when microwave-driven discharge is utilized. When sufficient charged particles accumulate on the surface of microorganisms, they are able to form an electric field and thus alter protein structure. This change in protein structure results in the creation of pores in the membrane of microorganisms or inhibition of enzymatic activities (11). Ma *et al.* (23) demonstrated cytoplasm leakage via pores generated by plasma-induced agents by detecting K^+ , proteins, and nucleic acids. Reactive species have considered play a crucial role in the inactivation

Table 1. Pathogen counts (Log CFU/g) in inoculated chicken breast samples following treatment with flexible thin-layer dielectric barrier discharge plasma for different time

Time (min)	Pathogens (Log CFU/g)		
	<i>Listeria monocytogenes</i>	<i>Escherichia coli</i> O157:H7	<i>Salmonella</i> Typhimurium
0	5.88 ^{a2)}	5.84 ^a	5.48 ^a
2.5	5.34 ^b	4.68 ^b	4.17 ^b
5	4.81 ^c	4.02 ^c	3.58 ^c
7.5	4.37 ^d	3.54 ^d	3.23 ^c
10	3.74 ^e	3.11 ^e	2.77 ^d
SEM ¹⁾	0.034	0.073	0.114

¹⁾Standard error of the mean ($n=15$).

^{2)a-c}Different letters within same column differ significantly ($p < 0.05$).

of microorganisms (6,12,18,23,25). Joshi *et al.* (26) reported that reactive species generated by plasma cause oxidative DNA damage and membrane lipid peroxidation.

Surface color The L^* (lightness), a^* (redness), b^* (yellowness) values of plasma-treated chicken breasts were significantly different compared with the untreated samples ($p < 0.05$) (Table 2). Subsequent to FTDBD plasma treatment, the L^* and b^* values of the chicken breasts increased with increasing treatment time ($p < 0.05$). In contrast, the a^* value was significantly decreased by plasma treatment compared with untreated chicken breasts ($p < 0.05$). Jayasena *et al.* (6) reported decreased a^* values and increased b^* values in beef loins treated with FTDBD plasma. A decrease in a^* value was also detected in pork butt samples. Another study also reported that plasma-treated pork (*Musculus longissimus dorsi*) presented decreased a^* values and increased b^* value (17). Greenish color of meat can be related with formation of sulfmyoglobin, choleglobin, and verdohaem (27). If hydrogen sulfide and oxygen react with myoglobin, sulfmyoglobin can be formed (17). Oxidized porphyrin ring develops green color by forming choleglobin and verdohaem (27). Fröhling *et al.* (17) demonstrated that the green color in plasma-treated meat sample is likely to be formed by hydrogen peroxide which is generated during plasma treatment.

TBARS values TBARS values are indicative of the level of lipid oxidation, which is a complex mechanism between unsaturated fatty acids and oxygen molecules (28) resulting in extensive quality

Table 2. Surface color and TBARS values of chicken breasts treated with flexible thin-layer dielectric barrier discharge plasma for different times

Time (min)	L^*	a^*	b^*	TBARS (mg malondialdehyde/kg meat)
0	55.78 ^d	-0.21 ^{a2)}	8.42 ^b	0.27
2.5	59.62 ^d	-1.93 ^{ab}	6.83 ^b	0.28
5	64.61 ^c	-2.82 ^b	6.00 ^b	0.26
7.5	74.22 ^b	-3.05 ^b	9.43 ^b	0.32
10	82.18 ^a	-1.31 ^{ab}	14.79 ^a	0.34
SEM ¹⁾	1.400	0.695	1.617	0.033

¹⁾Standard error of the mean ($n=15$).

^{2)a-d}Different letters within the same column differ significantly ($p < 0.05$).

Table 3. Texture profile analysis of chicken breasts treated with flexible thin-layer dielectric barrier discharge plasma for different times

Time (min)	Hardness (kg)	Adhesiveness (g/sec)	Springiness (mm)	Cohesiveness (%)	Gumminess (kg)	Chewiness (kg)
0	14.38	-31.02	0.71	0.40 ^{ab2)}	5.83	1.18
2.5	11.62	-23.67	0.72	0.36 ^b	4.37	0.95
5	13.58	-36.92	0.77	0.41 ^{ab}	5.69	1.23
7.5	16.73	-52.04	0.78	0.42 ^{ab}	7.23	1.36
10	17.13	-39.26	0.73	0.46 ^a	8.21	1.76
SEM ¹⁾	2.508	10.351	0.035	0.023	1.308	0.281

¹⁾Standard error of the means (n=15).

^{2)a-b}Different letters within the column differ significantly (p<0.05).

Table 4. Sensory evaluation of chicken breasts treated with flexible thin-layer dielectric barrier discharge plasma for different times

Time (min)	Sensory parameter					
	Appearance	Color	Flavor	Taste	Off-Flavor	Acceptability
0	5.00	4.84	5.22 ^{a2)}	5.16	1.36 ^{ab}	5.22
2.5	5.13	4.97	5.19 ^{ab}	4.88	1.53 ^{ab}	5.06
5	4.94	5.13	4.66 ^c	4.66	1.28 ^b	5.28
7.5	5.19	5.19	4.69 ^{bc}	4.75	1.72 ^a	5.47
10	4.78	5.08	4.75 ^{abc}	5.09	1.47 ^{ab}	5.06
SEM ¹⁾	0.178	0.144	0.168	0.197	0.126	0.230

¹⁾Standard error of the means (n=40).

^{2)a-c}Different letters within the column differ significantly (p<0.05).

deterioration in muscle tissue based food (29). Lipid oxidation is triggered by radicals (7), which are a component of plasma. Regardless of plasma treatment time, FTDBD plasma did not affect lipid oxidation in chicken breasts (Table 2). Kim *et al.* (8) obtained similar results for bacon samples treated with atmospheric pressure plasma using helium and helium/oxygen as carrier gases. However, Jayasena *et al.* (6) reported that lipid oxidation was accelerated by FTDBD plasma in pork butt and beef loin samples. Thus, TBARS values might be influenced by plasma treatment conditions including plasma type, carrier gas, and sample characteristics such as fat content and composition. TBARS value can be increased by an increase of fat content (30). Chicken breast has a lower total fat content (%) than beef or pork (*longissimus dorsi* and *semimembranosus* muscles) (31). In addition, the ferric heme pigment is considered to be a critical pro-oxidant in tissue (29) and TBARS values closely correlate with total pigment and myoglobin content (32). Chicken breast has lower heme iron content, pigment, and the ultimate metmyoglobin levels compared with pork and beef (32).

Texture Cohesiveness was the only texture analysis parameter affected in plasma-treated chicken breasts (Table 3). The level of cohesiveness increased with plasma exposure time (p<0.05). Using FTDBD plasma to inactivate microorganisms did not result in significant changes in any of the texture parameters of the pork butt and beef loin samples (6). There was no major effect on texture by plasma treatment. Irradiation, which is one of the nonthermal sterilization methods, have similarity. Since superoxide radicals, hydrogen peroxide, and singlet molecular oxygen act as main factors in bacterial inactivation (33). Irradiation treatment also have not

significant effect on texture in turkey breast roll (34).

Sensory evaluation To investigate the effect of FTDBD plasma on chicken breast, sensory evaluation was conducted (Table 4). Plasma treatment did not affect most sensory parameters evaluated except for flavor. Flavor score was slightly lower and the off-flavor score was higher when plasma exposure time was increased (p<0.05).

Lipid and protein oxidation caused by radicals produce secondary oxidation products such as aldehydes, alkanes, alkenes, hydrocarbons, ketones, alcohols, esters, and acids (7) several of these oxidation products have unique aromas that can influence the flavor of the original sample (28). Yong *et al.* (22) reported FTDBD plasma treatment of sliced cheddar cheese resulted in lower scores in flavor scores and higher off-flavor scores due to lipid oxidation. However, the change in flavor and off-flavor in the present study are most likely not caused by lipid oxidation since there were no significant changes in the TBARS values following plasma treatment. In addition, there were no changes in off-flavor in FTDBD treated pork butt and beef loin samples, although lipid oxidation was observed (6). Therefore, further studies are required to elucidate the factors that cause off-flavor in plasma treated chicken.

Mutagenicity assay The results presented in Table 5 indicate that 10 min plasma treatment at doses of up to 5,000 µg/plate did not affect mutagenicity. Maron and Ames (21) determined that when the number of revertant colonies is greater than that in the negative control, the sample is positive for mutagenicity. The number of revertant colonies in our positive control was 20 and 3-fold higher than our experimental samples with the TA98 and TA100 strains,

Table 5. *Salmonella* mutagenicity assay for chicken breasts treated with flexible thin-layer dielectric barrier discharge plasma for 10 min

Sample	Time	Dose ($\mu\text{g}/\text{plate}$)	Number of revertant colonies (His ⁺) ¹ per plate			
			TA98 (-S9)	TA98 (+S9)	TA100 (-S9)	TA100 (+S9)
Chicken breasts	0	1,250	21 \pm 7	32 \pm 1	345 \pm 44	358 \pm 48
		2,500	28 \pm 8	33 \pm 8	385 \pm 18	404 \pm 6
		5,000	22 \pm 3	28 \pm 4	416 \pm 25	402 \pm 19
	10	1,250	22 \pm 5	34 \pm 3	317 \pm 30	350 \pm 34
		2,500	17 \pm 2	35 \pm 1	340 \pm 39	328 \pm 40
		5,000	21 \pm 5	34 \pm 8	322 \pm 40	358 \pm 31
Negative control	EtOH ²		20 \pm 3	24 \pm 5	304 \pm 15	356 \pm 25
Positive control	4-NQO	0.5	1063 \pm 14			
	2-AA ¹	2		2055 \pm 95		
	SA	0.5			861 \pm 88	
	2-AA ²	2				2343 \pm 112

¹Values are the mean \pm SD ($p < 0.05$).

²EtOH, ethanol; 4-NQO, 4-Nitroquinoline-1-oxide; SA, Sodium azide; 2-AA¹, 2-Aminoanthracene dissolved in DDW; 2-AA², 2-Aminoanthracene dissolved in DMSO

respectively, indicating that the experiment was performed properly. No difference was found between plasma-treated and untreated chicken breasts and there was no dose-dependent response. The number of revertants per plate for the plasma-treated chicken breasts was the same as the negative control. This confirms the mutagenic safety of FTDBD plasma-treated chicken breast in sealed packages. Lee et al. (35) also conducted SOS chromotest to confirm the toxicological safety of using atmospheric pressure plasma jet in cooked egg whites and yolks.

From the results, our results indicate that FTDBD plasma can be applicable because of its ability to improve microbiological safety with minimal changes in the sensory properties of chicken breast.

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