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Effects of the treatment parameters on the efficacy of the inactivation of *Salmonella* contaminating boiled chicken breast by in-package atmospheric cold plasma treatment

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ABSTRACT

The effects of surface coating, microbial loading, surface-to-volume ratio, sample stacking, mixing of samples with romaine lettuce, and shaking of the samples on the inactivation of *Salmonella* contaminating boiled chicken breast (BCB) cubes using in-package atmospheric dielectric barrier discharge cold plasma (ADCP) treatment at 38.7 kV were investigated. Whey protein coating increased the ADCP treatment efficacy in inactivating *Salmonella* on BCB cubes; the *D*-value increased from 0.2 to 1.3 min when the initial inoculum concentration increased from 3.8 to 5.7 log CFU/sample. ADCP decontaminated stacked BCB samples uniformly, and shaking during the treatment increased the inactivation rate. The concentrations of chicken protein isolate, water, and soybean oil in a chicken breast model food that resulted in the highest *Salmonella* reduction (1.7 log CFU/sample) were 20.5%, 68.9%, and 10.6%, respectively. ADCP treatment did not affect the color and tenderness of the model food, irrespective of its composition. The present study indicated that ADCP is a feasible technology to decontaminate prepackaged ready-to-eat meat cube products.

1. Introduction

Microorganisms on chicken meat are mostly inactivated through cooking during the manufacture of cooked chicken products. However, microorganisms that survive the cooking process or those transferred via post-process contamination may grow on the products (Samelis et al., 1998). Pathogenic microorganisms, such as *Salmonella* spp., are frequently associated with disease outbreaks related to the consumption of cooked chicken products (Guard-Petter, 2001). Since 2007, 123 salmonellosis outbreaks resulting from the consumption of cooked chicken have been reported (CDC, 2018). Thus, the development of a new intervention technology to decontaminate cooked chicken products contaminated with *Salmonella* during manufacture is needed.

An atmospheric dielectric barrier discharge cold plasma (ADCP) actuator is generally composed of two metal electrodes and a dielectric barrier. When the electrical potential difference between the two electrodes reaches a certain level, a multitude of micro-discharges are produced by the dielectric barrier (Misra et al., 2016), which ionize the gas inside the reactor, thus generating reactive species, including charged particles, radicals, neutral species, and photons, that have antimicrobial effects (Arjunan et al., 2015; Min et al., 2017). Once a plastic-packaged food product has been exposed to ADCP, the food package itself may act as a dielectric barrier, implying that the ADCP treatment may be useful as an in-package decontamination method for food packaged in plastic containers (Min et al., 2017). In-package decontamination of food products is highly desirable for the food industry because it can eliminate post-process contamination (Min et al., 2017). Nonetheless, there are some challenges to the industrial adoption of ADCP as a food processing technology, including effective process control and validation, determination of the inactivation kinetic parameters, scale-up, and regulatory approval (Barba et al., 2017; Cullen et al., 2018).

Recently, ADCP has been used for inactivating microorganisms contaminating raw chicken. Lee et al. (2016) reported that when raw, skinless chicken breast inoculated with *Listeria monocytogenes*, *Escherichia coli*, and *S.* Typhimurium was treated with ADCP at 2 W, 15 kHz for 10 min, the microorganisms were inactivated by 2.1, 2.7, and 2.7 log CFU/g, respectively. Dirks et al. (2012) reported that, when ADCP (0.5 kHz, 30 kV, 3 min) was applied to raw skinless chicken breast, *L. monocytogenes* and *Campylobacter jejuni* were inactivated by 2.8 and 3.0 log CFU/g, respectively. Positive effects of ADCP treatment for decontaminating bulk romaine lettuce or grape tomatoes have also been reported, suggesting the potential use of ADCP treatment for microbial inactivation of packaged ready-to-eat (RTE) food products (e.g.,

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salad products) (Min et al., 2017, 2018). Nonetheless, ADCP treatment has not yet been systematically investigated as a decontamination method for cooked chicken RTE products. Contaminated RTE meat products have been identified as high-risk food products (Rød et al., 2012). Thus, developing an ADCP technology for decontaminating prepackaged RTE chicken breast would be relevant to the food industry.

The need for investigating the influence of packaging parameters, such as food package volume ratio, to realize ADCP as an in-package decontamination technology has been emphasized (Min et al., 2017). The potential for using ADCP treatment for decontaminating prepackaged RTE chicken breast needs to be investigated, while verifying the individual effects of food product, packaging, and treatment variables, including surface coating, microbial loading, surface-to-volume ratio, sample stacking, mixing of samples with romaine lettuce, and shaking of the samples during treatment. In this study, we used romaine lettuce-mixed RTE chicken breast as a model to evaluate ACDP treatment as a food safety intervention for decontaminating salad products containing chicken meat. Thus, the objectives of this study were to study the inactivation of *Salmonella* contaminating boiled chicken breast (BCB) using in-package ADCP treatment by investigating the effects of chicken surface uniformization using protein coating, microbial loading, surface-to-volume ratio, sample stacking, mixing of the BCB samples with romaine lettuce, and shaking of the samples during treatment on the inactivation efficacy. Furthermore, the composition of the food is one of the key factors influencing the effectiveness of microbial inactivation by intervention technologies (Butz and Tauscher, 2002). Thus, the effect of BCB composition on microbial inactivation was determined and the optimal composition resulting in the highest microbial inactivation was predicted. As the moisture and lipid contents in cooked chicken breast can vary, the effects of the composition of cooked chicken breast on the efficacy of ADCP treatment should be evaluated. Information obtained from this investigation may be useful for the food industry in guiding the application of ADCP with maximum efficacy for chicken meat decontamination.

2. Materials and methods

2.1. Materials

The chicken breast meat used in this study was a Nobrand (Dongwoo, Gunsan, Korea) product, which was stored at −20 ± 1 °C until use. Romaine lettuce was purchased pre-washed (Yongchun Union Farm, Chungju, Korea) from a local supermarket and was stored at 4 ± 1 °C for up to 3 days until use. Whey protein isolate (WPI) powder used as a coating base material and glycerol used as a plasticizer were obtained from BiPRO® (Davisco Foods International, Le Sueur, MN, USA) and Sigma-Aldrich (St. Louis, MO, USA). Chicken protein isolate (CPI, 87.5%) powder (BIN's 100% CHXN) and soybean oil (pure refined soybean oil) used for producing the chicken breast model food were purchased from BarnDad Innovative Nutrition LLC. (Pittsburgh, PA, USA) and Sajo Haepyo Corp. (Seoul, Korea), respectively.

2.2. Chicken breast meat sample preparation

Chicken breast meat was boiled in water at 100 °C for 90 min and then cut into cubes of 1.5 × 1.5 × 1.5 or 3.0 × 3.0 × 1.5 cm (width × length × height) using a razor sterilized in 70% (v/v) ethanol inside a laminar flow biohazard hood (Type A/B3; NuAire Inc., Plymouth, MN, USA). The chicken breast cubes were completely immersed in pre-prepared 10% (v/v) WPI coating solution for 3 min for coating, then dried in the biohazard hood for 2 h. The coating was repeated three times before the cubes were used in experiments. WPI coating solution was prepared as described by McHugh and Krochta (1994). A 10% (w/w) aqueous solution of WPI was prepared and heated in a water bath at 90 °C for 30 min for protein denaturation. Glycerol was added to the cooled solution (5%, w/w) to prepare the film-forming solution. To evaluate the effects of coating on ADCP treatment efficacy, uncoated chicken breast cubes were also prepared.

2.3. Romaine lettuce sample preparation

The outer (3–5) lettuce leaves were removed, and intact inner mature leaves were selected for the experiment. The lettuce leaves were cut to ~5 × 12 cm (2.0 g) using scissors sterilized with 70% (v/v) ethanol for estimating the inactivation of microorganisms and color. To reduce the background microbial load, the cut lettuce leaves were immersed in a sodium hypochlorite solution (300 mg/L, Yuhan-Clorox Co., Ltd., Seoul, Korea) for 3 min (Olanya et al., 2015), rinsed in sterilized deionized water five times, dewetted using a salad spinner, and dried in the biohazard hood at 22 ± 2 °C for 30 min prior to microbial inoculation. To evaluate the effects of ADCP treatment on leaf color, cut lettuce leaves were washed with running deionized water once and dried in the biohazard hood for 30 min and then used without further preparation.

2.4. Model food sample preparation

Using the central composite design of the response surface methodology (RSM; Minitab, ver. 16.0 Minitab Inc., State College, PA, USA), which has been successfully used to evaluate the effects of several variables and their interactions on response variables and optimize biotechnological processes related to food systems (Liyana-Pathirana and Shahidi, 2005), 13 compositions were produced on the basis of the contents of protein (31.0%, w/w), water (65.3%, w/w), and lipid (3.6%, w/w) of the chicken breast meat (USDA, 2018) (Table. 1). The model food was prepared in a biohazard hood according to the ingredient ratios shown in Table 1. CPI powder, distilled water, and soybean oil were weighed and then mixed using a sterilized reagent spoon. The mixture was wrapped with a cling film (Tapex Uniwrap Co., Hwasung, Korea) and sterilized with 70% ethanol. The wrapped mixture was stored at 4 ± 2 °C for 10 h and then cut into cubes of 1.5 × 1.5 × 1.5 cm using a knife sterilized with 70% (v/v) ethanol. The cubes were used as samples for determining microbial inactivation, color, and tenderness, without WPI coating.

Table 1
Experimental variables and their values for the determination of the optimum concentration of boiled chicken breast cube model food for inactivating *Salmonella* on the model food by atmospheric dielectric barrier discharge cold plasma treatment[1].

Sample number	Explanatory variables Water: X_1, C_1, soybean oil: X_2, C_2					Response variables
	Coded value		Real value		CPI[2] (%)	*Salmonella* reduction (log CFU/sample)
	X_1	X_2	C_1 (%)	C_2 (%)		
1	0	0	66.7	10.4	23.0	1.7 ± 0.1[a,3]
2	0	−1.4	73.8	0.8	25.4	0.9 ± 0.4[b]
3	0	0	66.7	10.4	23.0	1.6 ± 0.1[a]
4	−1	−1	65.8	4.6	29.6	1.1 ± 0.1[ab]
5	0	1.4	60.8	18.2	20.9	1.3 ± 0.3[ab]
6	1	−1	75.6	3.3	21.1	1.3 ± 0.3[ab]
7	0	0	66.7	10.4	23.0	1.6 ± 0.2[a]
8	1	1	67.2	14.0	18.7	1.5 ± 0.1[ab]
9	1.4	0	72.7	8.5	18.8	1.3 ± 0.4[ab]
10	0	0	66.7	10.4	23.0	1.3 ± 0.2[ab]
11	0	0	66.7	10.4	23.0	1.4 ± 0.3[ab]
12	−1	1	55.9	18.9	25.2	0.9 ± 0.3[b]
13	−1.4	0	57.1	13.3	29.5	1.2 ± 0.2[ab]

[1] The treatment voltage applied to the electrode, through the cold plasma reactor, and treatment time were 38.7 kV and 3.5 min, respectively.
[2] CPI: chicken protein isolate.
[3] The results are expressed as the mean ± standard deviations ($n = 9$). Values followed by different letters are significantly different ($p < 0.05$).

2.5. Microbial inoculation

The three strains of *Salmonella* used in the experiments were *S.* Typhimurium DT104, *S.* Montevideo (CCARM 8052), and *S.* Enteritidis (CCARM 8040). The *S.* Typhimurium DT104 strain was obtained from Food Science and Biotechnology Laboratory at the Agricultural Biotechnology Department of Seoul National University (Seoul, Korea), and *S.* Montevideo and *S.* Enteritidis were obtained from the Culture Collection of Antimicrobial Resistant Microbes (CCARM, Seoul Women's University, Seoul, Korea). Each strain was subcultured in tryptic soy broth (TSB; BD, Franklin Lakes, NJ, USA) in an incubator at 37 °C for 24 h. The cultures were centrifuged (10,000 rpm, 2 min) and the pellet was washed three times with 0.1% sterilized peptone water (BD). To prepare inoculum, the three strains of *Salmonella* were mixed at equal volumes and the concentration was checked through plating on xylose-lysine-desoxycholate agar (XLD; BD). In the experiment to study the individual effects of sample surface coating, microbial loading, and surface-to-volume ratio on *Salmonella* inactivation upon ADCP treatment, one side of the chicken breast sample was spotted with *Salmonella* inoculum (20 μL) at 8–12 locations, after which the inoculum was spread out using a sterile spreader (Lee et al., 2012). The *Salmonella* concentrations used for studying the effects of the coating and microbial loading were 4.4 ± 0.1 (WPI-non-coated) and 4.6 ± 0.2 (WPI-coated) log CFU/sample, respectively, and those used for investigating microbial loading effect were 3.8 ± 0.2, 4.6 ± 0.2, and 5.7 ± 0.2 log CFU/sample. To study the effect of stacking of the coated chicken breast samples on ADCP treatment efficacy in terms of *Salmonella* inactivation, the samples (± 15 samples for each time point) were immersed in 1 L of *Salmonella*-mixed strain inoculum (~7 log CFU/mL) for 5 min, with gentle stirring. The *Salmonella* concentration in the inoculated sample was 5.7 ± 0.3 log CFU/sample. In the experiment to study the microbial decontamination by ADCP treatment applied to mixed food containing chicken breast cubes and romaine lettuce, the chicken breast cubes and the romaine lettuce were immersed in 1 L of *Salmonella*-mixed strain inoculum (~7 log CFU/mL) for 5 min, and the *Salmonella* concentrations of the inoculated samples were 3.5 ± 0.3 and 2.8 ± 0.3 log CFU/cm^2, respectively. To study the effects of chicken breast composition on *Salmonella* inactivation upon ADCP treatment, *Salmonella* was inoculated at 4.2 ± 0.2 log CFU/sample by spot inoculation. All samples with and without inoculation were placed in the biohazard hood for ~2 h after inoculation prior to ADCP treatment.

2.6. Treatment sample preparation

Samples in different configurations (1.5 × 1.5 × 1.5 or 3.0 × 3.0 × 1.5 cm), either with or without inoculation with *Salmonella*, were transferred aseptically to a commercial clamshell container (100% polyethylene terephthalate (PET), 18.0 × 14.0 × 3.6 cm, DL-203; Dongyang D&P, Daegu, Korea) and the lid was closed inside the biohazard hood. The containers were sterilized with 70% ethanol and dried in the biohazard hood prior to being filled. To study the effects of WPI coating on *Salmonella* inactivation upon ADCP treatment, both coated and uncoated chicken breast cubes were prepared as samples, and one cube was placed in each clamshell container (Fig. 1a). To study the effects of initial microbial load on ADCP treatment efficacy, chicken breast cubes were WPI-coated and one cube was placed in each container. To study the effect of the container surface-to-volume ratio on ADCP treatment efficacy, four coated cubes of 1.5 × 1.5 × 1.5 cm (2 by 2) and one cube of 3.0 × 3.0 × 1.5 cm, with surface-to-volume ratios of 4.0 and 2.7 cm^{-1}, respectively, were prepared as samples (Fig. 1b), and to study the effects of sample stacking on ADCP treatment efficacy, a single layer of nine coated chicken breast cubes (3 by 3) and a double layer of thirteen coated chicken breast cubes (3 by 3, 2 by 2) were prepared and placed in one clamshell container (Fig. 1c). To study whether vegetable mixing affects *Salmonella* inactivation in cooked chicken samples, 8 cubes of chicken breast (4 by 2) and 3 pieces of romaine lettuce were prepared and placed in one clamshell container (Fig. 1d). In the experiment to study the effects of chicken breast composition on *Salmonella* inactivation, one chicken breast model food was placed in one clamshell container.

2.7. ADCP treatment system

The ADCP actuator generates a plasma field between the base dielectric barrier and the upper aluminum rectangular electrodes (Fig. 2). An AC power supply (220 V, 60 Hz) delivering a high voltage (60 Hz, up to 50 kV, peak-to-peak voltage) was coupled to the aluminum electrodes. The dielectric barrier was established by using a 3.5-mm-thick sheet of borosilicate glass (Seoul Glass Co., Ltd., Guri, Korea) of approximately 25 × 30 cm. The distance between the upper electrode and the borosilicate glass was 4 cm. The voltage was measured using a high-voltage electric probe (PN PVM-1; North Star, Marana, AZ, USA). The output of the probe was depicted on a digital oscilloscope (TDS-2024B, Tektronix, Beaverton, OR, USA).

2.8. ADCP treatment

To evaluate the effect of initial microbial load, samples inoculated with *Salmonella* at 3.8 ± 0.2 log CFU/sample were subjected to ADCP treatment for 0.3, 0.5, 1.0, and 1.5 min, while samples inoculated with *Salmonella* at 4.6 ± 0.2 and 5.7 ± 0.2 log CFU/sample were treated for 0.5, 1, 1.5, 2, and 2.5 min. The *D*-value was calculated by the following equation from data obtained from the first straight-line interval of the time-dependent survival curve drawn for each *Salmonella* inoculation concentration (Fernández et al., 2012):

$\log (N/N_0) = -t/D$, where N is microbial population at any time, N_0 is initial microbial population, and *D* is the decimal reduction time or time in minutes for the microbial CFU to be reduced by 1 log.

Except in the experiment studying the microbial loading effect, the samples were ADCP-treated for 3.5 min. All samples were ADCP-treated at 38.7 kV (kV_{RMS}). The ADCP treatment time (3.5 min) and voltage were selected based on a preliminary study as the conditions resulting in effective inactivation of *Salmonella* contaminating chicken breast without inducing arc discharges on the samples and container and altering meat color, among voltages of 10.0–38.7 kV and treatment periods of 0.5–5 min. The mixed food was shaken during the ADCP treatment. To this end, an insulation rod was fixed to the clamshell container and shaken at 2 turns/s for 15 s by hand at 45-s intervals. ADCP treatment was carried out under atmospheric pressure at 23 ± 2 °C and using air as the plasma-forming gas, and the temperature change inside the container immediately after the treatment of samples was 4 ± 1 °C.

2.9. Microbiological analysis

To investigate the effects of each variable (the application of sample coating, the initial concentration of *Salmonella* inoculated to the sample, the stacking of chicken breast samples, and the composition of the chicken breast model food) on ADCP treatment efficacy in terms of *Salmonella* inactivation, one sample was placed in a stomacher bag (384 mL, Nasco Whirl-Pak®, Fort Atkinson, WI, USA) for each microbiological analysis. In the experiment to study the effects of the surface-to-volume ratio, four chicken breast cubes of 1.5 × 1.5 × 1.5 cm or one cube of 3.0 × 3.0 × 1.5 cm were placed in a stomacher bag. In the experiment to study the effects of ADCP treatment on the mixed food product (8 chicken breast cubes and 3 romaine lettuce pieces), chicken and lettuce samples were separately placed in stomacher bags (each type in one bag). The chicken breast cubes or model food samples contained in the stomacher bags were mixed with 10 mL peptone water (0.1%, w/w), and the bags were rubbed by hand for 3 min. The romaine lettuce leaves contained in the stomacher bag were mixed with 38 mL peptone water (0.1%, w/w), and the bag was rubbed by hand for 3 min.

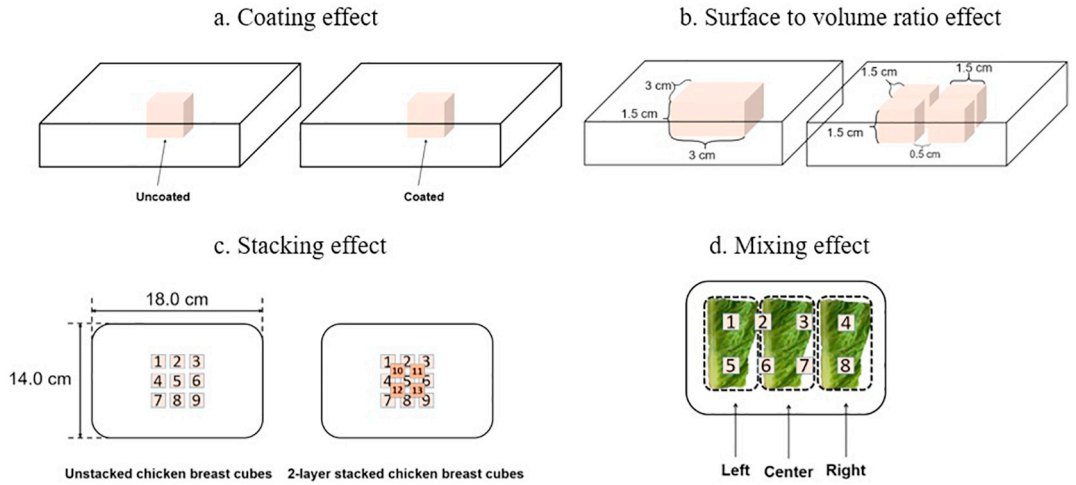

Fig. 1. Schematic diagram of chicken breast sample preparation for the experiments studying the effects of whey protein isolate coating, surface-to-volume ratio, number of chicken breast cubes, and mixing chicken breast samples with romaine lettuce on the inactivation of *Salmonella* on chicken breast cubes and romaine lettuce packaged in plastic packages by in-package atmospheric dielectric barrier discharge cold plasma treatment.

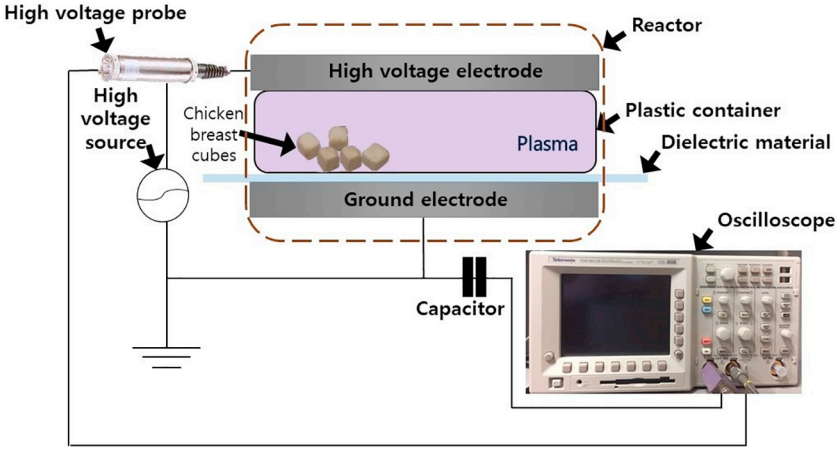

Fig. 2. Schematic diagram of the in-package atmospheric dielectric barrier discharge cold plasma system.

The resulting suspension was serially diluted in 0.1% peptone water. The diluted samples were plated on XLD agar and the XLD plates were incubated for 24 h at 37 °C for enumeration of *Salmonella*.

2.10. Physicochemical properties

2.10.1. Color

Color was determined with a colorimeter (Minolta Chroma Meter CR-400; Minolta Camera Co., Osaka, Japan) using Illuminant D65, 10° standard observer, and CIELAB scale (CIE L^* (lightness), a^* (redness) and b^* (yellowness)). The color of chicken breast cubes and model food samples was determined from the top and the four sides. The color of romaine lettuce was determined from five points on each leaf.

2.10.2. Tenderness

The tenderness of chicken breast meat was determined following the method of Barbanti and Pasquini (2005) using a texture analyzer (TA-XT2; Stable Micro System Co. Ltd., Surrey, UK) equipped with a Warner–Bratzler blade (HDP/BS; Stable Micro Systems) to determine the peak shear force (g). Test speed and cutting distance were set at 2.0 mm/s and 100%, respectively.

2.11. Statistical analysis

All experiments were performed in triplicate. For microbiological analysis and color determination, two samples were analyzed per repetition, and for tenderness estimation, five samples were analyzed per repetition. Means were compared using one-way analysis of variance, and Tukey's multiple range test was conducted in cases with a significant difference ($\alpha = 0.05$). Statistical analysis was conducted using SPSS (ver. 23.0.0; IBM SPSS Inc., New York, NY, USA).

The relationships among CPI powder, water, and soybean oil in the model food in terms of *Salmonella* reduction was analyzed using response surface analysis (RSA; Minitab), which resulted in the following secondary regression model:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2$$

where b_n is a regression coefficient; Y is the *Salmonella* reduction (log CFU/sample); X_1, X_2, and X_3 are the concentrations of the CPI powder (%, w/w), water (%, w/w), and soybean oil (%, w/w), respectively.

The ratio of ingredients exhibiting the strongest effects on *Salmonella* reduction was determined using the response optimizer function in Minitab. Pearson's correlation analysis (SAS version 9.4, SAS Institute Inc., Cary, NC, USA) was conducted to analyze correlations among the color or tenderness of the model food, treated or untreated with ADCP, and the concentrations of CPI powder, water, and soybean oil.

3. Results and discussion

3.1. Effects of sample surface coating on Salmonella inactivation by ADCP treatment and the color and texture of the chicken breast cubes before and after ADCP treatment

ADCP treatment inactivated *Salmonella* inoculated onto WPI-coated chicken breast cubes by 1.5 ± 0.3 log CFU/sample, which was a significantly higher rate than the inactivation of 0.8 ± 0.3 log CFU/sample in non-coated chicken breast cubes ($p < 0.05$). The effectiveness of cold plasma treatment for microbial inactivation is influenced by the inherent surface characteristics of the commodity being treated (Min et al., 2016). Potential irregularities on the chicken sample surface, including cracks, grooves, and pits, might induce shadow effects for ADCP-derived reactive species. We hypothesize that the WPI coating smoothened the surfaces of the chicken breast cubes, thereby allowing effective inactivation of the *Salmonella*. The results in this study suggest that ADCP treatment is an effective microbial decontamination technique for processed meat products with smooth surface, which may include film-coated food products, such as sausages.

Reactive species generated during ADCP treatment are very short-lived, but highly reactive. Thus, it is necessary to assess the effects of plasma treatment on physical properties, such as color (Noriega et al., 2011). According to Segat et al. (2015), reactive oxygen species (ROS), such as ozone, OH, HO_2, O^{2-} and O^{3-}, can react with the aromatic rings of the amino acid residues of WPI, which may enhance the yellow color. Moreover, plasma created in the presence of oxygen leads to the formation of ROS that may react with the amino-acid side chains of muscle proteins and thus cross-link proteins (Lund et al., 2011). The potential structural changes in chicken breast proteins induced by ADCP might result in altered tenderness of the meat. However, ADCP treatment under the conditions used in this study did not bring about significant changes in color of the WPI coating nor to the CIE L^*, a^*, and b^* values and tenderness of the chicken samples ($p > 0.05$; Table 2). These findings indicate that the ROS mostly reacted with the microorganisms on the sample surfaces, and remaining ROS were insufficient to induce color and texture changes. The absence of changes in texture profiles of raw chicken breast, pork butt, and beef loin after cold plasma treatment has been reported previously (Jayasena et al., 2015; Lee et al., 2016).

Table 2
Effects of in-package atmospheric dielectric barrier discharge cold plasma (ADCP) treatment[1] on color and tenderness of whey protein isolate (WPI)-coated or non-coated boiled chicken breast cubes.

Parameter		Sample		Value
Color	L^*	Uncoated	Untreated	82.23 ± 0.42[a,2]
			ADCP-treated	82.68 ± 0.36[a]
		WPI-coated	Untreated	80.90 ± 0.63[a]
			ADCP-treated	80.19 ± 1.19[a]
	a^*	Uncoated	Untreated	3.17 ± 0.26[a]
			ADCP-treated	3.03 ± 0.29[a]
		WPI-coated	Untreated	3.19 ± 0.31[a]
			ADCP-treated	3.28 ± 0.33[a]
	b^*	Uncoated	Untreated	12.96 ± 0.27[a]
			ADCP-treated	12.80 ± 0.36[a]
		WPI-coated	Untreated	14.46 ± 0.72[a]
			ADCP-treated	14.61 ± 0.47[a]
Tenderness (g)		Uncoated	Untreated	1354.02 ± 165.14[a]
			ADCP-treated	1599.34 ± 256.35[a]
		WPI-coated	Untreated	1682.21 ± 239.27[a]
			ADCP-treated	1530.50 ± 242.98[a]

[1] The treatment voltage applied to the electrode, through the cold plasma reactor, and treatment time were 38.7 kV and 3.5 min, respectively.

[2] The results are expressed as the mean ± standard deviations ($n = 9$). Values followed by different letters are significantly different ($p < 0.05$).

3.2. Effects of initial microbial load on ADCP treatment efficacy

When the initial microbial load on chicken breast was 3.8, 4.6, and 5.7 log CFU/sample, upon ADCP treatment, the *D*-value was 0.2, 0.8, and 1.3 min, respectively. This indicated that a low initial microbial load led to relatively more effective *Salmonella* inactivation, which is in accordance with a previous study by Fernández et al. (2012), who reported that increasing the concentration of *S*. Typhimurium inoculated onto a membrane filter decreased the ADCP treatment efficacy. When the concentration of microorganisms is high, the cells on the sample surface may form a multi-layer, small cluster, island, or web (Fernández et al., 2012), which secludes a fraction of the cells from direct exposure to ADCP treatment, which is a surface treatment (penetration depth: ≤10 nm, Morent et al., 2011), thereby lowering the microbial inactivation rate (Fernández et al., 2012). Moreover, when ADCP treatment fails to adequately decontaminate samples, cultivable microorganisms that were exposed to the plasma but escaped the inactivation may activate a detoxification system that generates proteins that quench the plasma-derived reactive species. Through these proteins, the cells may acquire resistance to cold plasma; hence, the decreased inactivation rate (Fernández et al., 2012). The results in this study confirmed that the initial microbial load must be taken into account when evaluating the effectiveness of ADCP treatment.

The temperature of the samples determined immediately after ADCP treatment never exceeded 29 °C (ΔT: 4.0 ± 1.0 °C), suggesting that the treatment inactivated *Salmonella* on the samples in a non-thermal manner.

3.3. Effects of surface-to-volume ratio on ADCP treatment efficacy

Salmonella was uniformly inactivated by 1.4–1.6 log CFU/sample regardless of the surface-to-volume (2.7 or 4.0 cm^{-1}) ($p > 0.05$). As ADCP treatment is a surface treatment (Deilmann et al., 2008), its effectiveness needs to be viewed in relation to the surface-to-volume ratio of the food (Hertwig et al., 2015). When the surface-to-volume ratio is high, chances are higher that reactive species reach non-inoculated sides of the food sample, resulting in less efficient microbial inactivation than in products with a low surface-to-volume ratio (Kim et al., 2014). A significant effect of the surface-to-volume ratio on the inactivation of *Bacillus cereus* spores inoculated on red pepper flake samples using microwave-powered cold plasma treatment was previously reported (Kim et al., 2017). A higher reduction in the number of *B. cereus* spores was observed in a flake sample with a ratio of 76 cm^{-1} (estimated using 3.0 mm as the flake thickness) (1.4 log spores/cm^2) than in a sample with a ratio of 70 cm^{-1} (0.8 log spores/cm^2). The absence of a significant effect of the ratio in the current study might be because of the smaller surface-to-volume ratios of the samples or a smaller difference between the ratios of the samples compared. Nonetheless, it may be suggested that when the effect of the surface-to-volume ratio of the food on the treatment efficacy is evaluated, the kinds of microorganisms, food products, and cold plasma treatment need to be considered.

3.4. Effects of chicken breast stacking on the ADCP treatment efficacy

ADCP treatment uniformly inactivated *Salmonella* in chicken breast samples configured in a single layer and those stacked in double layer, with no significant difference in microbial inactivation rates among the different sample positions ($p > 0.05$; Table 3). The levels of inactivation in non-stacked (single layer) samples and in stacked (double layer) samples were 1.5 ± 0.5 and 1.8 ± 0.4 log CFU/sample, respectively,

Table 3
Effects of in-package atmospheric dielectric barrier discharge cold plasma treatment[1] on the inactivation of *Salmonella* on stacked or non-stacked chicken breast cubes samples.

Sample number designated in Fig. 1c	Microbial reduction (log CFU/sample)	
	Unstacked sample	Stacked sample
1	1.6 ± 0.1[a,2]	1.6 ± 0.2[a]
2	1.5 ± 0.2[a]	1.9 ± 0.4[a]
3	1.3 ± 0.4[a]	1.8 ± 0.1[a]
4	1.5 ± 0.7[a]	1.7 ± 0.4[a]
5	1.5 ± 0.4[a]	1.9 ± 0.5[a]
6	1.7 ± 0.9[a]	1.8 ± 0.4[a]
7	1.6 ± 0.5[a]	2.0 ± 0.3[a]
8	1.6 ± 0.3[a]	1.9 ± 0.4[a]
9	1.5 ± 0.5[a]	1.5 ± 0.0[a]
10	–	1.8 ± 0.6[a]
13	–	2.0 ± 0.6[a]

[1] The treatment voltage applied to the electrode, through the cold plasma reactor, and treatment time were 38.7 kV and 3.5 min, respectively.
[2] The results are expressed as the mean ± standard deviations ($n = 9$). Values followed by different letters are significantly different ($p < 0.05$).

Table 5
Effects of atmospheric dielectric barrier discharge cold plasma treatment[1] on the inactivation of *Salmonella* on chicken breast cubes and romaine lettuce at designated positions in the mixed food.

Sample (numbers designated in Fig. 1d)	Microbial reduction (log CFU/cm^2)
Chicken breast cube 1	1.4 ± 0.4[a,2]
Chicken breast cube 2	1.2 ± 0.1[a]
Chicken breast cube 3	1.2 ± 0.3[a]
Chicken breast cube 4	1.4 ± 0.2[a]
Chicken breast cube 5	1.6 ± 0.2[a]
Chicken breast cube 6	1.4 ± 0.3[a]
Chicken breast cube 7	1.4 ± 0.4[a]
Chicken breast cube 8	1.5 ± 0.3[a]
Romaine lettuce left	1.3 ± 0.1[a]
Romaine lettuce center	1.5 ± 0.1[a]
Romaine lettuce right	1.2 ± 0.1[a]

[1] The treatment voltage applied to the electrode, through the cold plasma reactor, and treatment time were 38.7 kV and 3.5 min, respectively.
[2] The results are expressed as the mean ± standard deviations ($n = 9$). Values followed by different letters are significantly different ($p < 0.05$).

which were not significantly different ($p > 0.05$). According to Min et al. (2017), *E. coli* O157:H7 contaminating the topmost layer in a 7-layer romaine lettuce was inactivated more effectively than microbes on the other layers of the stack ($p < 0.05$), which was attributed to the concentrated action of the reactive species generated by cold plasma on the topmost layer of romaine lettuce, while even spreading was prohibited by the stacking. However, in this study, similar levels of inactivation were observed for all positions of chicken breast cubes in a single or double layer. This might be because the space between the package lid and the top of the chicken sample was large enough for plasma-derived reactive species to spread.

When the influence of ADCP treatment on the color of chicken breast cubes was monitored, no color change was detected in both single- and double-layer samples, regardless of the position of the cubes ($p > 0.05$; Table 4). Product color may be affected by cold plasma treatment due to the presence of reactive species in the plasma, which are capable of oxidizing and degrading colorants (Min et al., 2017). Furthermore, ozone generally present in ADCP can lead to bleaching of the food (Misra et al., 2014). The absence of discoloration in this study might be because insufficient reactive species were generated to induce color change in BCB or because of the WPI coating that might reduce the chance of reaction between reactive species and the meat. Effects on color have been reported in fresh produce and animal origin foods, although the results differ according to the plasma treatment and the food tested (Pignata et al., 2017).

The lack of significant changes in the samples irrespective of the configuration and position of the cubes revealed that ADCP treatment affected the samples in a uniform manner.

3.5. Effects of ADCP treatment on Salmonella inactivation in the mixture food containing chicken breast cubes and romaine lettuce

ADCP treatment without shaking led to 1.2–1.6 log CFU/cm^2 inhibition of *Salmonella* inoculated onto the chicken breast cubes in the mixture, regardless of the position of each cube ($p > 0.05$; Table 5). This is in agreement with the results obtained in the experiment using 9 or 13 chicken breast cubes ($p > 0.05$; Table 3). Thus, the mixing of romaine lettuce with chicken breast did not affect the microbial decontamination efficacy. ADCP treatment reduced the number of *Salmonella* on romaine lettuce by ~1.4 log CFU/cm^2, regardless of the position of the sample, which was not significantly different from the inactivation rate on chicken breast cubes ($p > 0.05$).

When shaking was integrated in the treatment, the *Salmonella* inactivation rates on chicken breast cubes and lettuce samples was reduced by 2.8 ± 0.2 log CFU/cm^2 and 1.3 ± 0.1 log CFU/cm^2,

Table 4
Effects of in-package atmospheric dielectric barrier discharge cold plasma (ADCP) treatment[1] on the color of non-stacked chicken breast cubes.

Sample number designated in Fig. 1c	L^*		a^*		b^*	
	Untreated	ADCP-treated	Untreated	ADCP-treated	Untreated	ADCP-treated
1	80.36 ± 2.21[a,2]	80.17 ± 2.08[a]	1.28 ± 1.03[a]	1.05 ± 0.83[a]	14.53 ± 1.27[a]	14.66 ± 1.48[a]
2	80.50 ± 1.38[a]	80.26 ± 1.17[a]	1.59 ± 0.79[a]	1.42 ± 0.72[a]	14.17 ± 1.74[a]	14.26 ± 1.76[a]
3	80.88 ± 1.54[a]	80.95 ± 1.57[a]	1.69 ± 1.51[a]	1.53 ± 1.29[a]	14.20 ± 0.95[a]	14.36 ± 1.05[a]
4	80.16 ± 2.44[a]	79.87 ± 2.24[a]	1.71 ± 0.83[a]	1.69 ± 0.79[a]	14.26 ± 1.14[a]	14.50 ± 1.12[a]
5	80.80 ± 1.73[a]	80.46 ± 1.67[a]	1.11 ± 0.57[a]	1.01 ± 0.60[a]	13.77 ± 1.64[a]	13.96 ± 1.40[a]
6	80.29 ± 1.37[a]	80.20 ± 1.06[a]	1.55 ± 0.91[a]	1.39 ± 0.92[a]	13.75 ± 1.46[a]	14.09 ± 1.06[a]
7	80.06 ± 1.45[a]	80.29 ± 1.33[a]	2.21 ± 0.55[a]	1.70 ± 0.39[a]	14.12 ± 1.51[a]	14.26 ± 1.53[a]
8	80.27 ± 1.77[a]	80.18 ± 1.34[a]	1.64 ± 1.13[a]	1.41 ± 0.99[a]	14.38 ± 0.88[a]	14.51 ± 0.92[a]
9	80.26 ± 1.09[a]	80.19 ± 1.09[a]	1.75 ± 1.02[a]	1.48 ± 0.98[a]	13.89 ± 1.04[a]	14.06 ± 1.44[a]

[1] The treatment voltage applied to the electrode, through the cold plasma reactor, and treatment time were 38.7 kV and 3.5 min, respectively.
[2] The results are expressed as the mean ± standard deviations ($n = 9$). Values followed by different letters in the same property are significantly different ($p < 0.05$).

Table 6
Effects of in-package atmospheric dielectric barrier discharge cold plasma treatment[1] without shaking on the color of romaine lettuce samples in a mixture of chicken breast cubes and romaine lettuce leaves.

Sample positions (designated in Fig. 1d)	L^*		a^*		b^*	
	Untreated	ADCP-treated	Untreated	ADCP-treated	Untreated	ADCP-treated
Left	43.57 ± 3.14^{a,2}	42.86 ± 1.95^{b}	−17.04 ± 1.02^{a}	−16.59 ± 1.50^{b}	27.62 ± 2.34^{a}	27.32 ± 2.19^{b}
Center	43.55 ± 2.05^{a}	41.99 ± 2.15^{b}	−17.11 ± 0.90^{a}	−16.32 ± 1.33^{b}	27.22 ± 1.84^{a}	26.14 ± 2.28^{b}
Right	43.53 ± 1.98^{a}	40.75 ± 2.60^{b}	−17.26 ± 0.45^{a}	−16.33 ± 1.20^{b}	27.45 ± 1.08^{a}	26.07 ± 1.45^{b}

[1] The treatment voltage applied to the electrode, through the cold plasma reactor, and treatment time were 38.7 kV and 3.5 min, respectively.
[2] The results are expressed as the mean ± standard deviations ($n = 9$). Values followed by different letters in the same property are significantly different ($p < 0.05$).

respectively, irrespective of their positions in the mixture. For the meat cubes, the integration of shaking increased the *Salmonella* inactivation rate ($p < 0.05$). The higher microbial inactivation rate upon shaking may be because of a better distribution of the ADCP-derived reactive species throughout the package, increasing the exposure of individual samples. Nonetheless, shaking did not affect the efficacy of *Salmonella* inactivation on the lettuce samples ($p > 0.05$). As previously described, it is difficult to achieve effective inactivation by cold plasma when the food contains porous or complex surface structures (Min et al., 2016; Raballand et al., 2008). Therefore, the potentially more complex surface structure of romaine lettuce in comparison to WPI-coated chicken breast cubes might have limited the increase in treatment efficacy.

The color of the lettuce leaves was significantly changed after ADCP treatment of the mixed food without shaking ($p < 0.05$; Table 6). The change in color may be explained by the fact that the romaine lettuce has relatively sharp edges in comparison to the coated chicken breast cubes. Arc was continuously formed at certain edges of the lettuce leaves and the color of the edges where arc was formed was altered.

The CIE L^*, a^*, and b^* for romaine lettuce in the left side of the package during shaking were 43.4 ± 2.24, −16.49 ± 1.06, and 26.51 ± 2.04, respectively; for romaine lettuce in the center were 42.46 ± 2.02, −16.94 ± 0.83, and 26.18 ± 1.92; and for romaine lettuce on the right were 43.91 ± 1.96, −16.67 ± 1.33, and 25.49 ± 1.35. Under shaking during ADCP treatment, the color of the lettuce leaves was not changed ($p < 0.05$). Continuous formation of arc on lettuce edges was prevented by shaking, which could lead to insignificant color change. Thus, the integration of shaking in the ADCP treatment improved the treatment efficacy in a mixture food containing chicken breast and romaine lettuce, without causing changes in color characteristics.

3.6. Effects of ADCP treatment on a chicken breast model food

3.6.1. Salmonella inactivation

Table 1 presents the effects of ADCP treatment on the inactivation of *Salmonella* inoculated onto chicken breast model food produced with varying ingredient concentrations on the basis of a central composite design. The reaction surface model equation for *Salmonella* reduction in the model food is as shown below:

$$Y = (2.755) + (-18.708)X_1 + (-34.615)X_2 + (-33.129)X_3 + (-58.958)X_1X_2 + (-27.794)X_1X_3 + (-25.088){X_1}^2 + (-26.555){X_2}^2 + (21.495){X_3}^2.$$

The model equation has a p value smaller than 0.0001, indicating that the variables are explained adequately by the equation. The p value for lack of fit was 0.31 and the R^2 value was 0.78, confirming that the data on *Salmonella* reduction in this study fitted adequately to the above model equation. A good fit between experimental data and the values predicted by the model is indicated by high R^2 (typically > 0.7) together with a p-value for the lack of fit higher than 0.05 (Delgado et al., 2018; Serrat et al., 2011). Nevertheless, the terms representing the concentrations of CPI powder (%, w/w), water (%, w/w), and soybean oil (%, w/w) and the term representing their interaction had p values of 0.217, 0.217, 0.220, 0.306, 0.337, 0.299, 0.362, and 0.379, which indicated none of the variables affected the *Salmonella* reduction. The optimum concentrations of CPI powder, water, and soybean oil in the model food to achieve maximum microbial inactivation were predicted as 20.5%, 68.9%, and 10.6%, respectively, and this composition was predicted to result in a *Salmonella* reduction of 1.7 log CFU/sample. Experimentally, ADCP treatment of the model food of optimum composition led to *Salmonella* reduction of 1.7 ± 0.2 log CFU/sample, which corresponded with the predicted value. The results confirmed the appropriateness of the experimental design using RSM for predicting *Salmonella* reduction by ADCP treatment in the model food.

The optimum concentration of CPI powder was relatively low in comparison with those used in the study while that of water was relatively high and that of soybean oil appeared to be moderate (Table 1). The results suggest that the microbial inactivation was favored at low protein and high moisture concentrations. High protein content may reduce the antimicrobial efficacy due to scavenging of plasma reactive species and allowing microorganisms to recover from the treatment (Bourke et al., 2018). Furthermore, the induction of water molecules evaporated from foods into the plasma-forming air in the package can produce antimicrobial reactive species, O, O_2^*, and hydrogen in the cold plasma, which is expected to enhance microbial inactivation (Min et al., 2016).

3.6.2. Color

The concentrations of CPI powder, water, and soybean oil in the model food, and their linear correlation with the color of the model food, are presented in Table. 7. Pearson's correlation analysis indicated that the lightness of the model food treated or not with ADCP was negatively correlated with CPI powder concentration, but positively correlated with water and soybean oil concentrations (Table 7). The enhanced lightness may be attributed to the dilution of CPI powder by the addition of liquids, i.e., water and soybean oil (Qiao et al., 2001). The redness and yellowness values of the model food treated or not with ADCP decreased as the concentrations of CPI powder and soybean oil decreased and the concentration of water increased (Table 7), possibly due to the dilution of CPI powder and soybean oil by the addition of water. This corresponds with the results of Ergönül (2017), where redness and yellowness values of raw chicken breast meat were lowered by increasing the concentration of water (%).

As described earlier, the various reactive species generated during ADCP treatment may bring about changes in food color by reacting with the food components (Min et al., 2017). The results of this study showed that ADCP treatment did not affect the color of the model food systems, irrespective of its composition based on CPI powder, water,

Table 7
Pearson correlation coefficient (r) values for color and tenderness of chicken breast model food with and without atmospheric dielectric barrier discharge cold plasma (ADCP) treatment[1].

Parameters	Color												Tenderness (g)			
	L^*				a^*				b^*				Untreated		ADCP-treated	
	Untreated		ADCP-treated		Untreated		ADCP-treated		Untreated		ADCP-treated					
	r	P	r	P	r	P	r	P	r	P	r	P	r	P	r	P
CPI[2] powder (%)	−0.873	< 0.0001	−0.911	< 0.0001	0.822	< 0.0001	0.830	< 0.0001	0.719	< 0.0001	0.697	< 0.0001	0.930	< 0.0001	0.873	< 0.0001
Water (%)	0.240	0.0066	0.337	< 0.0001	−0.725	< 0.0001	−0.711	< 0.0001	−0.712	< 0.0001	−0.715	< 0.0001	−0.288	0.0134	−0.256	0.0267
Soybean oil (%)	0.360	< 0.0001	0.361	< 0.0001	0.299	0.0412	0.267	0.0002	0.323	< 0.0001	0.344	< 0.0001	−0.336	0.0036	−0.300	0.0090

[1] The treatment voltage applied to the electrode, through the cold plasma reactor, and treatment time were 38.7 kV and 3.5 min, respectively.
[2] CPI: chicken protein isolate.

and soybean oil that were tested in this study ($p > 0.05$; Table 8). In addition, no significant alteration in color was observed when the model food of optimum composition for *Salmonella* inactivation was treated by ADCP ($p > 0.05$; Table 8).

3.6.3. Tenderness

The tenderness of the model food exhibited a positive correlation with CPI powder concentration, while it was negatively correlated with water and soybean oil concentrations (Table 7). This indicates that the mixture became softer with increasing proportion of the liquids (Zhuang and Savage, 2010).

ADCP treatment did not affect tenderness, irrespective of the model food composition ($p > 0.05$; Table 8), and no significant difference in tenderness was observed when the model food of optimum composition for *Salmonella* inactivation was treated or not with ADCP ($p > 0.05$; Table 8).

The composition of chicken breast meat may vary in terms of protein, water, and lipid contents depending on the chicken breed and the processing method. The protein, water, and lipid contents of the meat may affect its color and tenderness and the inactivation of microorganisms contaminating it by reacting with the reactive species generated during ADCP treatment. However, our results suggest that ADCP treatment would not affect the color and tenderness of the chicken breast meat having the protein, water, and lipid contents used in the model food system. In addition, *Salmonella* reduction occurred regardless of the model food composition, suggesting that the ADCP treatment used in this study will be efficacious for commercial chicken breast products.

4. Conclusions

WPI coating was helpful to effectively inactivate *Salmonella* contaminating chicken breast by smoothening its surface, without altering its color or tenderness. As the initial microbial load of *Salmonella* on chicken breast increased, the inactivation of *Salmonella* by ADCP treatment became increasingly less effective. The surface-to-volume ratio designed and tested in this study was found to not affect *Salmonella* inactivation during ADCP treatment of chicken breast. ADCP treatment was shown to uniformly inactivate *Salmonella* at all positions on the chicken breast, without altering its color, regardless of stacking. When shaking was integrated into the ADCP treatment, the *Salmonella* in the mixed food containing chicken breast and romaine lettuce was inactivated in a uniform manner, without altering product color. Shaking was also shown to significantly improve the *Salmonella* inactivation rate for chicken breast. The experiment using a model food revealed that the proportions of protein, water, and lipid influence *Salmonella* reduction by ADCP treatment. For the model food composition used in this study, ADCP treatment did not lead to significant changes in color or tenderness. The results from this study demonstrate that ADCP treatment mediates uniform microbial inactivation in stacked or non-stacked meat cube samples regardless of the position or surface-to-volume ratio of the samples and the presence of lettuce slices, which indicates that ADCP treatment may allow uniform microbiological decontamination of chicken breast meat cubes in salad products. Furthermore, ADCP treatment is expected to be effective for and applicable to chicken food products in the industry since the model food used in this study mimicked regular chicken breast. It is suggested that chicken breast contained in a salad product should be coated with an edible film prior to ADCP treatment for microbial inactivation, and that shaking should be ideally integrated into the ADCP treatment for the decontamination of packaged chicken breast salads.

Table 8
Effects of atmospheric dielectric barrier discharge cold plasma (ADCP) treatment[1] on the color and tenderness of chicken breast model food.

Sample number designated in Table 1	L^*		a^*		b^*		Tenderness (g)	
	Untreated	ADCP-treated	Untreated	ADCP-treated	Untreated	ADCP-treated	Untreated	ADCP-treated
1	77.27 ± 0.37^{aAB,2}	76.96 ± 0.60^{aCD}	1.56 ± 0.06^{aC}	1.55 ± 0.05^{aC}	15.45 ± 0.79^{aAB}	15.38 ± 0.86^{aC}	262.6 ± 11.9^{aCD}	351.8 ± 109.6^{aCDEF}
2	73.42 ± 0.50^{aAB}	73.15 ± 0.43^{aF}	1.32 ± 0.05^{aDE}	1.30 ± 0.06^{aDE}	14.81 ± 0.69^{aAB}	14.70 ± 0.56^{aDE}	516.4 ± 87.4^{aB}	584.3 ± 84.9^{aB}
3	77.55 ± 0.30^{aAB}	77.33 ± 0.21^{aC}	1.47 ± 0.20^{aCD}	1.47 ± 0.20^{aCD}	15.00 ± 0.21^{aAB}	14.99 ± 0.22^{aCD}	389.8 ± 36.7^{aBC}	435.7 ± 41.4^{aBCD}
4	71.46 ± 11.55^{aB}	73.07 ± 1.66^{aF}	2.42 ± 0.07^{aA}	2.43 ± 0.09^{aA}	17.17 ± 0.27^{aAB}	16.96 ± 0.35^{aB}	1024.3 ± 184.6^{aA}	1112.0 ± 152.7^{aA}
5	76.56 ± 11.49^{aAB}	79.26 ± 1.25^{aA}	1.21 ± 0.05^{aE}	1.16 ± 0.07^{aEF}	15.60 ± 1.53^{aAB}	14.98 ± 0.86^{aCD}	245.0 ± 138.1^{aCD}	268.5 ± 53.5^{aDEF}
6	75.81 ± 0.43^{aAB}	76.21 ± 0.70^{aDE}	0.64 ± 0.13^{aG}	0.61 ± 0.12^{aH}	13.34 ± 0.60^{aB}	13.35 ± 0.84^{aF}	232.6 ± 22.2^{aCD}	243.3 ± 22.3^{aEF}
7	77.14 ± 0.59^{aAB}	77.14 ± 0.73^{aCD}	1.58 ± 0.07^{aC}	1.55 ± 0.07^{aC}	15.36 ± 0.57^{aAB}	15.36 ± 0.61^{aC}	362.9 ± 87.8^{aBC}	424.7 ± 71.0^{aBCD}
8	78.54 ± 0.52^{aA}	78.76 ± 0.49^{aA}	1.13 ± 0.06^{aEF}	1.10 ± 0.09^{aF}	14.77 ± 0.47^{aAB}	14.60 ± 0.64^{aDE}	162.8 ± 33.1^{aD}	193.5 ± 38.5^{aF}
9	76.09 ± 12.23^{aAB}	78.59 ± 0.82^{aAB}	0.94 ± 0.16^{aF}	0.87 ± 0.20^{aG}	14.28 ± 0.62^{aB}	14.31 ± 0.90^{aE}	201.3 ± 22.3^{aCD}	181.5 ± 18.2^{aF}
10	77.95 ± 0.75^{aAB}	77.72 ± 0.58^{aBC}	1.58 ± 0.09^{aC}	1.59 ± 0.08^{aC}	15.44 ± 0.30^{aAB}	15.59 ± 0.47^{aC}	295.9 ± 55.3^{aCD}	348.7 ± 81.1^{aCDEF}
11	77.72 ± 0.59^{aAB}	77.73 ± 0.73^{aBC}	1.50 ± 0.15^{aCD}	1.47 ± 0.17^{aCD}	15.53 ± 0.27^{aAB}	15.52 ± 0.28^{aC}	335.2 ± 16.8^{aBCD}	377.3 ± 4.1^{aCDE}
12	76.09 ± 0.59^{aAB}	75.81 ± 1.39^{aE}	2.07 ± 0.09^{aB}	2.03 ± 0.07^{aB}	16.70 ± 0.30^{aA}	16.90 ± 0.34^{aB}	391.1 ± 70.4^{aBC}	407.9 ± 100.1^{aCDE}
13	73.69 ± 1.01^{aAB}	73.55 ± 1.17^{aF}	2.41 ± 0.14^{aA}	2.44 ± 0.21^{aA}	17.80 ± 0.62^{aAB}	17.60 ± 0.65^{aA}	487.6 ± 25.9^{aB}	492.9 ± 35.5^{aBC}

[1] The treatment voltage applied to the electrode, through the cold plasma reactor, and treatment time were 38.7 kV, and 3.5 min, respectively.
[2] The results are expressed as the mean ± standard deviations ($n = 9$). Values represented by different superscript letters in the same column (lowercase letter) or in the same row (uppercase letter) are of the same property are significantly different ($p < 0.05$).

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