Effect of plasma-activated acetic acid on inactivation of *Salmonella* Typhimurium and quality traits on chicken meats

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ABSTRACT This study investigated the bactericidal effects of plasma-activated acetic acid (**PAAA**) on *Salmonella* Typhimurium and its impact on the physicochemical traits of chicken meat. Twenty milliliters of 0.8% (v/v) acetic acid (**AA**) was treated with plasma (2.2 kHz and 8.4 kVpp) for 30 min. The chicken skins, breasts, and drumsticks, inoculated with *S.* Typhimurium, were immersed in AA or PAAA and incubated for 10 min. The *S.* Typhimurium on the breasts and drumsticks were significantly susceptible to treatment with AA and PAAA, compared to the control group (deionized water treatment), and the population of bacterial cells in PAAA-treated chicken breasts and drumsticks decreased by 0.98 and 1.19 log CFU/g, respectively, compared with AA. The values for pH and 2-thiobarbituric acid reactive substances (**TBARS**) of PAAA-treated samples decreased significantly compared to the control group. The lightness (L^*) values of the chicken breasts after AA and PAAA treatments increased compared to the control group, whereas the value for yellowness (b^*) decreased. The scanning electron microscopic (**SEM**) images and the results for volatile compounds in chicken meat revealed similar patterns, with no significant differences between AA and PAAA treatments. In conclusion, we found that PAAA was more effective than AA and synergistic PAAA treatment of chicken caused to the reduction of *S.* Typhimurium and improve the meat quality. Therefore, PAAA could be utilized as a promising decontaminant for the chicken meat industry.

Key words: plasma-activated acetic acid, chicken meat, *Salmonella* Typhimurium, bactericidal efficiency, meat quality

INTRODUCTION

With the increasing consumption of meat and meat products, the number of foodborne pathogen outbreaks related to meat has significantly increased (Zhao et al., 2001). Meat and meat products are highly susceptible to contamination by foodborne pathogens such as *Salmonella* spp., *Campylobacter* spp., Shiga toxin-producing strains of *Escherichia coli,* and *Listeria monocytogenes,* during their production, processing, and transportation (Nerin et al., 2016; Omer et al., 2018; Lee et al., 2021b). Particularly, chicken meat is a highly perishable product because of its characteristics that can cause rapid and intensive spoilage (Noriega et al., 2011). Most of the pathogen contamination in chicken meat can occur in slaughterhouses through spread of microorganisms between carcasses (Kim et al., 2019). A previous study reported that *Salmonella* spp. account for majority of the foodborne pathogens identified in poultry and poultry products (Dominguez et al., 2002). Therefore, many chicken meat industries face problems in the effective inactivation of *Salmonella* spp. as well as in ensuring that the quality of chicken meat is maintained. However, the inactivation of pathogens and deterioration of the quality of chicken meat remains a significant challenge (Dirks et al., 2012). Numerous efforts have been made to inactivate microbial contaminants in chicken meat using thermal treatments, use of bacteriocins or lactic acid bacteria, and washing with agents such as chlorine and trisodium phosphate (Mani-López et al., 2012). However, these traditional methods have some limitations in inactivating pathogens and adversely affect the nutritional value or sensory quality of chicken meat (Whyte et al., 2001; Kim et al., 2002; Berrang et al., 2007).

In many countries, organic acids such as acetic, citric, and lactic acid, which are designated by the European committee, FAO/WHO, and FDA as generally

recognized as safe substances (**GRAS**) for meats (Surekha and Reddy, 2000), are commonly used for the decontamination of chicken meat industries due to their antimicrobial potency, cost-effectiveness, and application simplicity (Cosansu and Ayhan, 2010). The antimicrobial traits of organic acids are based on their ability to lower the pH, thereby causing instability in the bacterial cell membranes (Reis et al., 2012). With the increasing consumer demand that industries reduce their use of chemical additives, many meat industries want to control the amount of organic acids used and simultaneously increase their antimicrobial effect in meat products (Sagong et al., 2011).

Recently, nonthermal technologies such as ultrasound, irradiation, ultra-high pressure, and pulsed light have been developed as alternatives to traditional methods (Heo et al., 2021). Cold plasma has a minimal impact on the quality of meat and meat products, is relatively inexpensive, and easy to install compared to other nonthermal technologies (Lee et al., 2016). In the present study, we prepared plasma-activated acetic acid (**PAAA**) by treating acetic acid with plasma (**AA**) to improve the bactericidal efficiency of AA. Some studies on LA treated with plasma have studied the synergistically bactericidal effects of meat (Qian et al., 2019, 2020). However, no study has been reported on the synergistically antibacterial effects of PAAA and the impact on the physicochemical traits of chicken meat.

The antibacterial effect and mechanism of acidic solution, such as acetic acid, obtained by treating plasma has also not been studied yet. Generally, reactive oxygen and nitrogen species (**RONS**) with a long half-life, such as H_2O_2, NO_2^- and NO_3^- may be the major antimicrobial agents and mediate a series of complex chemical reactions in plasma-activated water (**PAW**; Samukawa et al., 2012). However, Oehmigen et al. (2010) suggested that the synergistic action of RONS is potentiated under acidic conditions, which leads to enhanced antibacterial activity. Thus, the pH value affects the activity of reactive species in plasma-activated liquid. Additionally, NO_2^- and H_2O_2 contribute to the formation of peroxynitrous acid (ONOOH), which is highly cytotoxic under acidic conditions (Lukes et al., 2014). During plasma discharge, H_2O_2 is utilized for the NO_2^- dependent generation of ONOOH under acidic conditions (Lukes et al., 2014; Laurita et al., 2015; An et al., 2019). ONOOH is a powerful oxidant that can diffuse through cell membranes, damage cells, and promote cell death through apoptosis can necrosis (Huie and Padmaja, 1993; Denicola et al., 1998). Considering the result that AA among organic acids has the highest sterilization efficiency against *Salmonella* Typhimurium (Mani-López et al., 2012), it is important to investigate the combined impacts on PAAA as a potential means for improving the efficiency of antibacterial activity. Our study could help to provide an understanding of the quality changes of chicken in the process of PAAA as a novel approach. Therefore, the objective of this study was to investigate the antibacterial effects of PAAA against *S.* Typhimurium and its impact on the quality characteristics of chicken meat.

MATERIALS AND METHODS

Bacterial Strains and Culture Preparation

S. Typhimurium (ATCC 13311) was obtained from the Korean Culture Center of Microorganisms (Seoul, Korea). *S.* Typhimurium was cultivated at 37°C in nutrient broth (Difco, Becton Dickinson Co., Sparks, MD) for 48 h to obtain mid-log phase cells. The strain was then washed twice with 0.85% NaCl solution (saline), followed by centrifugation at 2,266 × *g* for 14 min at 2°C (UNION 32R, Hanil Science Industrial, Co. Ltd, Gimpo, Korea). Finally, the viable cell density of the re-suspended culture was adjusted to approximately 10^8 to 10^9 CFU/mL using the optical density at a wavelength of 600 nm.

Sample Preparation, Sterilization, and Inoculation

Raw chicken skin, breasts, and drumsticks were purchased in advance from a local market in Seoul (Nonghyup Co., Seoul, Korea) and frozen at −20°C. Before the experiment, the samples were thawed overnight (24 h) at 4°C. The skin was punched into 2 cm² round pieces, and the breasts and drumsticks were cut into equal-sized pieces (3.00 ± 0.05 g) using a sterile knife. To study the antibacterial effect, the surface of each sample was exposed to ultraviolet light for 30 min to eliminate background microflora. Hundred microliters of the cell suspension were spot inoculated at nine different points on the surface of the samples and spread with a sterile spreader for even distribution and attachment. The samples were then placed for 1 h on a clean bench at room temperature (25°C) to allow bacterial adsorption to the surface of the samples.

Preparation of PAAA

As presented in Figure 1, an encapsulated atmospheric dielectric barrier discharge plasma, using a rectangular plastic container (137 × 104 × 53 mm) containing copper electrodes and a polytetrafluoroethylene sheet, was prepared for the generation of PAAA. Atmospheric air was used as the operating gas and plasma was generated at 2.2 kHz and 8.4 kVpp following the modified conditions from a previous study (Yoo et al., 2021). The AA immersed in a glass dish was
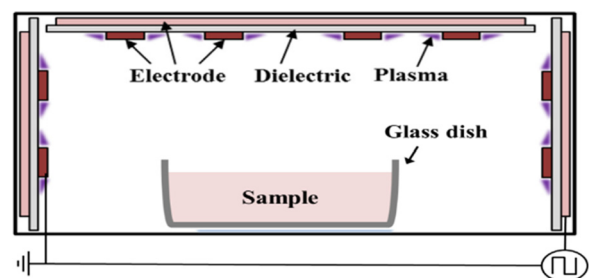

Figure 1. Schematic diagram of the experimental setup for the preparation of dielectric barrier discharge plasma.

placed in the center of the container, and then exposed to plasma generated inside the container. PAAA was obtained by exposing the plasma discharge to 20 mL of AA (v/v) for 30 min. The concentrations of AA used in this study were 0.2, 0.6, and 0.8% (v/v), representing the corresponding AA concentrations in PAAA, respectively. The selected concentrations were based on preliminary studies. The bacterial cells in the samples treated with deionized water were used as a control (Royintarat et al., 2020). Additionally, based on our preliminary study, there was no significant effect on the detachment of bacterial cells of being washed away by immersing method.

Microbial Analysis

To investigate the bactericidal effect of bacteria, inoculated samples were immersed in 50 mL conical tubes containing AA and PAAA for each treatment and incubated at 25°C for 10 min (Royintarat et al., 2020). Immediately after plasma treatment, each sample was placed in a new tube containing 27 mL of saline solution. Each sample in tube was vigorously vortexed at high speed for 2 min for the detachment of bacteria on the surface of chicken meats. After the detachment of bacteria, the supernatants from each tube were serially diluted (1:10) in saline. Each diluted sample (0.1 mL) was spread on Xylose Lysine Deoxycholate agar (Difco) and agar plates were incubated at 37°C for 48 h for the determination of $S.$ Typhimurium counts. The results of the skin were expressed as log CFU/cm^2, and the breasts and drumsticks were expressed as log CFU/g. The number of colony forming units per round pieces cm of the chicken skin, called the density, is found by Equation (1) (Hamilton et al., 2003).

$$\text{Density} = \frac{Avg\ count}{Drop\ volume} \text{Dilution} \qquad (1)$$
$$\cdot \text{Volume scraped into} \frac{1}{Surface\ area}$$

Where Avg count is the average of the raw data counts [CFU], Drop volume is the volume of the drop plated [mL], Dilution is the $1/10^{-k}$ where k is an integer for 10-fold dilutions, Volume scraped into is the volume of the liquid the chicken skin was scraped into [mL], Surface area is the scraped surface area of the chicken skin [cm^2].

pH Measurement

The pH values of the breasts and drumsticks were measured according to a previously described method (Heo et al., 2021). After each treatment, 1 g of the homogenized sample was added to each tube containing 9 mL distilled water and mixed thoroughly for 30 s using a homogenizer (T25 Basic, Ika Co., Staufen, Germany). After homogenization, the solution was centrifuged (Hanil Science Industrial Co., Ltd.) at 2,265 × g for 10 min at 4°C and the pH value of the resulting supernatant was measured using a pH meter (Seven 2Go,

Mettler-Toledo International Inc., Schwerzenbach, Switzerland).

Lipid Oxidation Measurement

The lipid oxidation of the breasts and drumsticks was analyzed according to a previously reported method, which calculates the level of 2-thiobarbituric acid reactive substances (**TBARS**) (Lee et al., 2016). Three grams of each sample and 9 mL of distilled water were mixed and homogenized with 50 μL of butylated hydroxytoluene (7.2% in ethanol) using a homogenizer at 9,600 rpm for 30 s. The homogenate (1 mL) was then added to a 15 mL centrifuge tube containing 2 mL of TBA (20 mmol/L)/trichloroacetic acid (15%) solution, and the tubes were heated in a water bath at 90°C for 30 min, followed by cooling in water. The test tubes were centrifuged at 2,265 × g for 10 min, and the absorbance of the supernatant was measured at 532 nm using a spectrophotometer (DU 530; Beckman Instruments Inc., Brea, CA). The TBARS value was presented as mg of malondialdehyde per kg sample, using a standard curve (Yim et al., 2020).

Color Measurement

The color of the breasts and drumsticks was measured using a colorimeter (CM-5, Konica Minolta Co., Ltd., Osaka, Japan) to obtain the CIE lightness ($\boldsymbol{L^*}$), redness ($\boldsymbol{a^*}$), yellowness ($\boldsymbol{b^*}$), hue angle ($\tan^{-1}(b^*/a^*)$), and chroma ($(a^{*2} + b^{*2})^{1/2}$). The instrument was calibrated with a white and black standard tile before the analysis (Yoo et al, 2020). Measurements were taken randomly on the surface of the breasts and drumsticks at 6 different locations per sample with an 8 mm diameter measurement area. The color values were monitored by a computerized system using spectra Magic software (Konica Minolta Sensing, Inc.).

EM Analysis

The scanning electron microscopic (**SEM**) images of the breasts and drumsticks were obtained following the method reported in our previous study (Shin et al., 2020). After each treatment, the samples were cut into 0.5 cm diameter and 0.2 to 0.3 cm thick pieces and were fixed with Carnoy fluid (60% ethyl alcohol, 30% chloroform, 10% glacial acetic acid; v/v) at 4°C for 24 h. The samples were dehydrated using an increasing concentration of ethyl alcohol (70% for 12 h, 95% for 2 h, and 100% for 2 h). Each dehydrated sample was immersed in hexamethyldisilazane twice for 10 min and dried overnight (24 h) in a fume hood at 25°C. The dried samples were mounted on aluminum stubs using double-sided carbon tape and coated with a layer of platinum in a vacuum evaporator (EM AC E600, Leica Microsystem, North Ryde, NSW, Australia). Micrographs of the samples were visualized using a Zeiss Sigma field emission

scanning electron microscope (AURIGA, Carl Zeiss Microscopy, Thornwood, NY).

Electronic Nose Analysis

The electronic nose (Heracles II, Alpha MOS, Toulouse, France) in each treatment was analyzed to determine the effect of PAAA on the odor of the treated chicken meat. The samples were ground using a meat grinder (MG510, Kenwood, Hampshire, UK), and each sample (5.00 ± 0.05 g) was taken in a 20-mL vial and cooked for 30 min at 80°C to obtain the volatile compounds (Li et al., 2020). The volatiles were then injected into an electronic nose equipped with dual columns of MXT-5 and MXT-1701 (10 m × 180 m × 0.4 m; length × diameter × thickness) (Restek, Bellefonte, PA). Each peak was integrated and identified using the retention time and relevance index, indicating the percentage of matching probability, based on the comparison of Kovats retention index of the detected compound and the Kovats retention indices of known compounds from the AroChemBase library (Lee et al., 2019).

Statistical Analysis

All experimental procedures were conducted independently in triplicates. The data were assessed by Tukey's multiple-range test using the SAS program (version 9.4, SAS Institute Inc., Cary, NC) at a significance level of $P < 0.05$. Statistical analysis was performed using the Student's t test and one-way analysis of variance. The standard deviation of the mean values is reported in the figures and tables. Scores plot based on principal component analysis (**PCA**) was generated using MetaboAnalyst 4.0, in accordance with the method mentioned by Kim et al. (2020).

RESULTS AND DISCUSSION

Antibacterial Effect of PAAA on Chicken Meats

The population of bacterial cells in both AA and PAAA treatments significantly decreased with increasing concentrations of AA ($P < 0.05$, Figure 2A). The initial population of S. Typhimurium on the skin was 6.91 log CFU/cm^2. The population of S. Typhimurium decreased by 0.17, 1.05, and 2.33 log CFU/cm^2 after incubation for 10 min with 0.2, 0.6, and 0.8% PAAA, respectively. However, after treatment with AA, the population of S. Typhimurium decreased by 0.07, 0.93, and 1.23 log CFU/cm^2 under the same concentrations of AA (0.2, 0.6, and 0.8%), respectively.

The bactericidal efficiency of 0.6% PAAA ($P < 0.05$) and 0.8% PAAA ($P < 0.01$) were significantly higher than those of the control and 0.2% PAAA treatment groups. These data suggest that S. Typhimurium on chicken skin has a more significant susceptibility to PAAA treatment compared with that of AA, and it is significantly affected by the increasing concentration of AA. This provides a potential application in the slaughtering process. The reactive species in chemical reactions generated by plasma discharge induce lethal effects on bacteria due to the role of these short-lived species (Zhou et al., 2015). For solutions that were treated with plasma, the antibacterial effect of PAW is attributed to the acidic pH and ONOOH generated by H_2O_2 and NO_2^- (Naïtali et al., 2010; Oehmigen et al., 2011). Qian et al. (2019) also found that the number of S. Enteritidis inoculated on beef significantly decreased with increasing concentration of lactic acid in plasma-activated lactic acid (**PALA**), which can accelerate the generation of NO_2^-. Further, NO_2^- induces the generation of ONOOH with H_2O_2. Similarly, it showed the same trend in our study that the low pH values of 0.8% PAAA and the concentrations of H_2O_2 and NO_3^- increased significantly in 0.8% PAAA with the increase in plasma discharge time ($P < 0.05$) (data not shown). These findings may indicate that the formation of increased ONOOH, especially in liquids with pH lower than 4. In addition, because of the low pH of PAAA, there are more potential possibility to form acidified nitrites, possess strong cell toxic properties (Babaeva et al., 2012). Thus, the antibacterial activity of PAAA is also more potent with increasing concentrations of AA. As shown in Figure 2A, when the concentration of AA was 0.8%, the bactericidal efficiency and the level of significance for differences between PAAA and AA treatment were the highest ($P < 0.01$). Therefore, 0.8% AA concentration was regarded as the optimal treatment condition and was used to investigate the antibacterial activity of the chicken breasts and drumsticks.

Figure 2B shows the antibacterial effect of 0.8% AA and PAAA on the breasts and drumsticks. Each sample was incubated for 10 min with 0.8% AA and PAAA obtained by plasma exposure for 30 min. After treatment with AA, the population of S. Typhimurium on the breasts and drumsticks significantly reduced by 1.35 and 1.56 log CFU/g, respectively, compared with the control group ($P < 0.05$). whereas, after treatment with 0.8% PAAA, the population of S. Typhimurium on the breasts and drumsticks significantly reduced by 2.33 and 2.75 log CFU/g, respectively ($P < 0.05$). These findings indicate that the susceptibility of the bacteria in chicken meat treated with PAAA was significantly higher than that treated with AA, which is consistent with the results of chicken skin presented in Figure 2A. According to a previous study, the efficacy of plasma was greatly reduced due to the surface topography on chicken skins, which may act as a physical barrier, resulting in the bacteria being protected from the reactive species generated by plasma, as compared to chicken muscle (Noriega et al., 2011). However, Rossow et al. (2018) identified that there were no significant differences in the bactericidal effects between the skin and breast samples by investigating the efficacy of parameters in plasma treatment. Consistent with this study, no significant differences in bactericidal effects
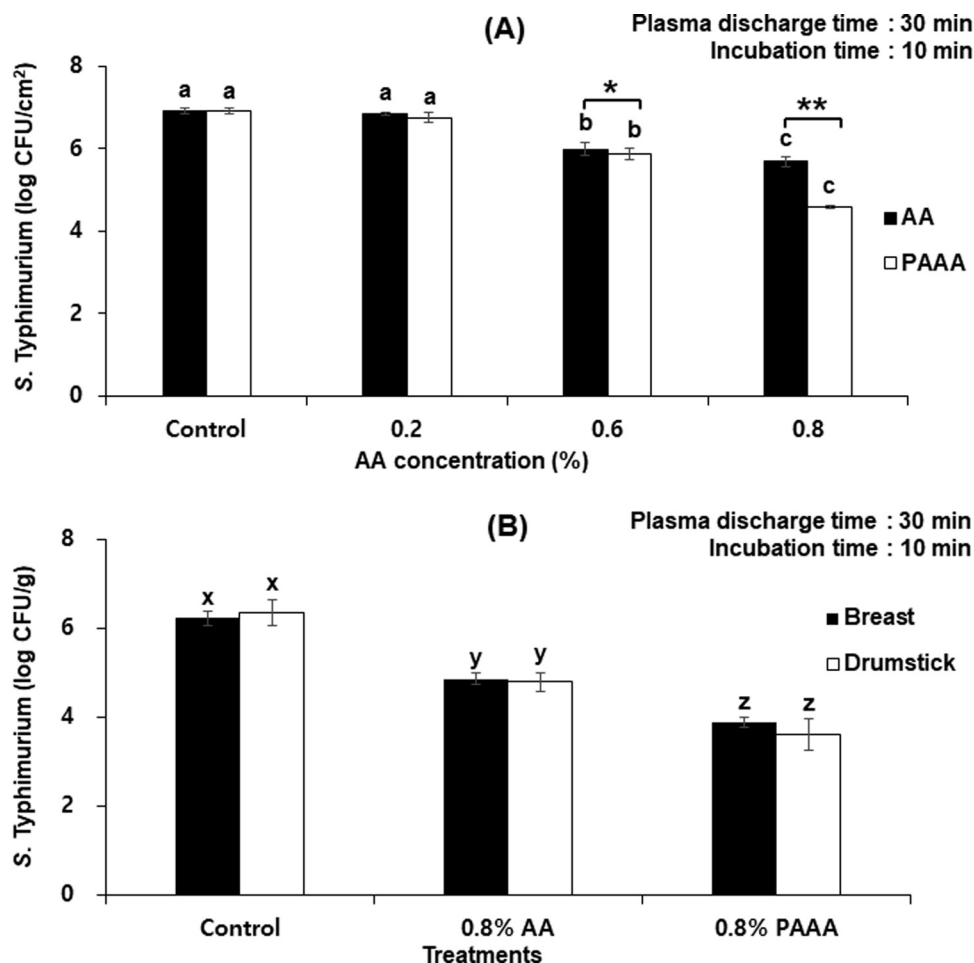
Figure 2. Effect of acetic acid (AA) and plasma-activated acetic acid (PAAA) on population of *Salmonella* Typhimurium according to (A) the concentration of AA on chicken skins and (B) the antibacterial effects of 0.8% AA and PAAA on chicken breasts and drumsticks. Control group, deionized water treatment; AA, AA treatment; PAAA, PAAA treatment. Error bars represent standard deviation. [a-c]Different letters indicate a significant difference ($P < 0.05$) among the treatments. [x-z]Different letters indicate a significant difference ($P < 0.05$) among the treatments. Student's t test; *, $P < 0.05$ and **, $P < 0.01$ with respect to the untreated control.

were observed between the skin and breast samples in our study. Therefore, these results suggest that *S.* Typhimurium can be effectively inactivated in chicken meat by treatment with PAAA.

pH

Table 1 presents the pH values of the breasts and drumsticks treated with AA or PAAA. The breasts and drumsticks showed significant differences between the control and treatment groups (0.8% AA and PAAA); the treatment group showed a decrease in pH values as compared to the control group. However, no significant differences were identified between the 0.8% AA and PAAA groups ($P > 0.05$). The decrease in the pH of chicken meat was related to the combined action of AA and PAAA. Aktaş et al. (2003) identified that the type and concentration of acids used significantly affected the pH values of marinated meat due to the dissociation of hydrogen ions (H^+). The plasma-induced decrease in pH values in the samples was caused by the generation of acidogenic molecules from the plasma, resulting in their accumulation on the surface of the samples

Fröhling et al., 2012). In addition, the interaction of the reactive species generated by plasma, including O, O_3, and NO_X, with the moisture content of the samples caused a decrease in the pH values in the samples after plasma treatment via Equations (2) to ((4) (Liu et al., 2010). However, it was found that there was no difference between AA and PAAA treatment groups in the

Table 1. pH and lipid oxidation of breasts and drumsticks treated with acetic acid (AA) and plasma-activated acetic acid (PAAA).

Treatments	pH	TBARS value (mg malondialdehyde /kg)
Breast		
Control	5.88 ± 0.01^a	0.27 ± 0.02^a
0.8% AA	5.28 ± 0.07^b	0.10 ± 0.01^b
0.8% PAAA	5.36 ± 0.18^b	0.10 ± 0.01^b
Drumstick		
Control	6.77 ± 0.03^a	0.31 ± 0.03^a
0.8% AA	5.90 ± 0.02^b	0.17 ± 0.02^b
0.8% PAAA	5.95 ± 0.08^b	0.19 ± 0.04^b

Control, deionized water treatment; 0.8% AA, acetic acid (0.80%, v/v) treatment; 0.8% PAAA, plasma-activated acetic acid (0.8%, v/v) treatment.

All values represent the Mean ± Standard Deviation.

[a,b]Different letters within column differ significantly ($P < 0.05$).

pH in the present study.

$$N_2 + O_2 \rightarrow 2NO \tag{2}$$

$$2NO + O_2 \rightarrow 2NO \tag{3}$$

$$2NO_2 + H_2O \rightarrow HNO_2 + HNO_3 \tag{4}$$
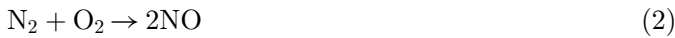
TBARS

The TBARS values of the treatments (0.8% AA and PAAA) in breasts and drumsticks were lower than those of the control group (Table 1). Significant differences in TBARS values of the breasts and drumsticks were observed between the control and treatment groups. Several studies have shown a decrease in the TBARS value of meat treated with organic acids, such as acetic, lactic, and citric acid, compared with the control group. Kang et al. (2002) reported that TBARS values of pork treated with acetic acid were lower than those of the control group during storage, and this finding was supported by the inhibition of the generation of malondialdehyde, which is induced by products such as carbonyl complexes, alcohols, ketones, and aldehydes. According to previous research, the TBARS values of jerky treated with dielectric barrier discharge plasma decreased as the plasma treatment time increased, which was caused by the antioxidant effect of nitrite (Yong et al., 2019). On the other hand, the TBARS values of pork treated with plasma were slightly higher than those of untreated pork sample, indicating the result might be different due to the variations in fat content and fatty-acid composition of sample (Jayasena et al., 2015). In the present study, there were no significant differences in TBARS values observed between the 0.8% AA and PAAA treatments ($P > 0.05$), suggesting that the action of plasma has little effect on 0.8% PAAA treatment. Similarly, the differences of TBARS values between lactic acid and PALA were insignificant, demonstrating that plasma-activated liquids didn't cause more severe lipid oxidation in beef samples (Qian et al., 2019).

Color

The color of meat is the most common indicator of quality, which has an important influence on consumer preferences (Wadhwani and Mcmahon, 2012; Kang et al., 2019). The L^* values of the breasts and drumsticks treated with 0.8% AA and PAAA increased, whereas the a^* and b^* values decreased compared to those of the control group (Table 2). However, there were no significant differences in color values (L^*, a^*, and b^*) between the control group and the treatments (0.8% AA and PAAA) in drumsticks. These results indicate that both samples had similar patterns in L^*, a^*, and b^* values, except for the significant differences. The color difference in meat is attributed to the amount of heme pigments and fibers type of predominant muscle (Berri et al., 2001). Lengerken et al. (2002) reported that breast meat

contains more than 90% white fibers, whereas the proportion of red fibers and irregular surface color in leg meat is higher than in other meat types. This could be the reason why there were no significant differences among the treatments in drumsticks compared to the breasts. Stivarius et al. (2002) identified that ground beef treated with AA was lighter ($P < 0.05$; L^*) and less red (a^*) and yellow (b^*) ($P < 0.05$) in color compared to the control group. In addition, the ground beef from the treatment with AA showed higher hue angle values than the control group. Similarly, in the present study, the hue angles in the breasts and drumsticks were higher than those of the control group. These results were related to the concentration of oxymyoglobin in meat, and could be attributed to the discolored meat, which caused lower redness values and oxymyoglobin content after treatment with AA (Bell et al., 1986; Stivarius et al., 2002).

According to a previous study, the L^* and b^* values of chicken breasts treated with dielectric barrier discharge plasma also increased as the treatment time increased, whereas the a^* value decreased significantly (Lee et al., 2016). Another study also identified significant increases in the L^* values and significant decreases in the a^* and b^* values were observed in breasts treated with PAW (Kang et al., 2019). Fröhling et al. (2012) demonstrated that the hydrogen peroxide generated in plasma induced a green color in plasma-treated meats *via* reaction with myoglobin. Consistent with the previous discussion, the color of breasts and drumsticks treated with 0.8% PAAA was additionally affected by the action of plasma, although there were no significant differences between the 0.8% AA and PAAA treatments ($P > 0.05$).

SEM

The skin was removed from the surfaces of breasts and drumsticks for each sample; these showed a smooth and regular shape on the SEM images (Figure 3). As shown in Figure 3, there were no remarkable morphological alterations observed after treatment with AA or PAAA. However, compared to the control group in each sample, the images of AA or PAAA treatment clearly revealed the presence of pores. These could be correlated to the porous structure of chicken meat, which is caused by the weakening of structures under acidic conditions (Alagöz et al., 2020). Royintarat et al. (2020) identified that the combined treatment of PAW and ultrasound was more porous with the surface of chicken meat compared with individual treatment, resulting in bacteria being susceptible to PAW treatment. However, Lin et al. (2019) reported that the SEM images of eggs revealed less damage to the cuticles on eggs treated with PAW than on the commercially washed eggs. In the present study, no additional morphological changes were observed on the surface of PAAA-treated chicken breasts and drumsticks compared with the AA treatment. Thus, the morphological changes on the surface of chicken meat may not be attributed to the action of plasma in the present study.

Table 2. Surface color values of breasts and drumsticks treated with acetic acid (AA) and plasma-activated acetic acid (PAAA).

Treatments	L^*	a^*	b^*	Chroma	Hue angle
Breast					
Control	51.33 ± 2.00^{b}	3.15 ± 0.14^{a}	13.65 ± 0.20^{a}	14.01 ± 0.22^{a}	77.09 ± 0.46
0.8% AA	55.93 ± 1.43^{a}	2.08 ± 0.43^{ab}	10.40 ± 0.20^{b}	10.65 ± 0.27^{b}	78.65 ± 2.17
0.8% PAAA	58.56 ± 0.32^{a}	1.50 ± 0.80^{b}	9.90 ± 0.06^{b}	10.04 ± 0.10^{b}	82.55 ± 5.77
Drumstick					
Control	52.02 ± 1.60	8.97 ± 1.53	15.05 ± 0.76	15.99 ± 0.84	61.17 ± 5.35
0.8% AA	51.65 ± 6.04	7.88 ± 0.37	14.68 ± 2.63	16.71 ± 2.13	61.33 ± 5.56
0.8% PAAA	53.13 ± 2.90	7.45 ± 2.38	13.82 ± 0.72	15.75 ± 2.03	63.16 ± 6.38

Control, deionized water treatment; 0.8% AA, acetic acid (0.80%, v/v) treatment; 0.8% PAAA, plasma-activated acetic acid (0.8%, v/v) treatment.
All values represent the Mean ± Standard Deviation.
L^*: Lightness; a^*: redness; b^*: yellowness.
[a,b]Different letters within column differ significantly ($P < 0.05$).

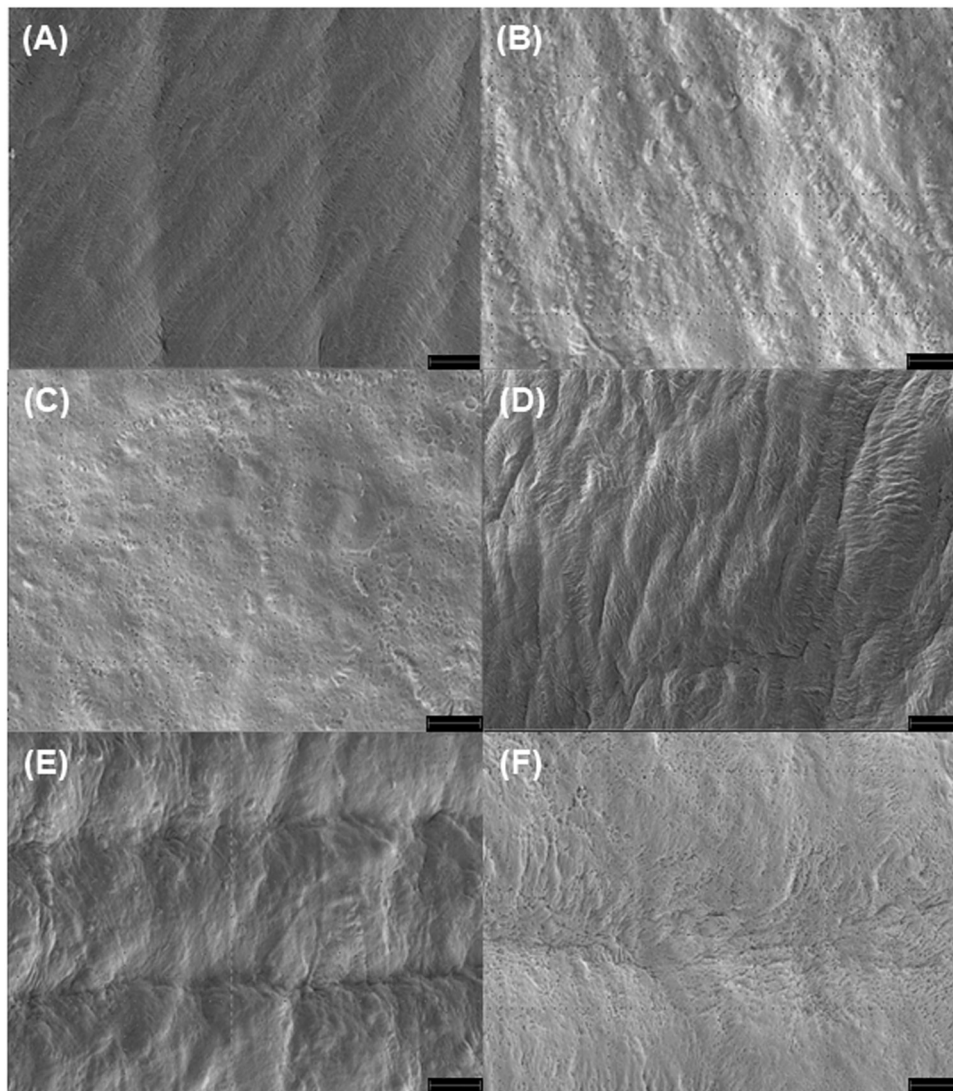**Figure 3.** Evaluation of morphological images of chicken meats using scanning electron microscope (SEM). (A) Control group (breasts) (× 2,000 magnification); (B) 0.8% acetic acid (AA) treatment (breasts) (× 2,000 magnification); (C) 0.8% plasma-activated acetic acid (PAAA) treatment (breasts) (× 2,000 magnification); (D) Control group (drumsticks) (× 2,000 magnification); (E) 0.8% AA treatment (drumsticks) (× 2,000 magnification); (F) 0.8% PAAA treatment (drumsticks) (× 2,000 magnification). Control, deionized water treatment; 0.8% AA, 0.8% AA treatment; 0.8% PAAA, 0.8% PAAA treatment. Bar, 10 μm.

Electronic Nose

Electronic nose is a rapid analysis tool to detect and distinguish between various types of gaseous samples (Chen et al., 2021). It is possible to obtain comprehensive information on volatile compounds in samples (Lee et al., 2021a). The PCA results obtained for the volatile compounds of chicken meat and are presented
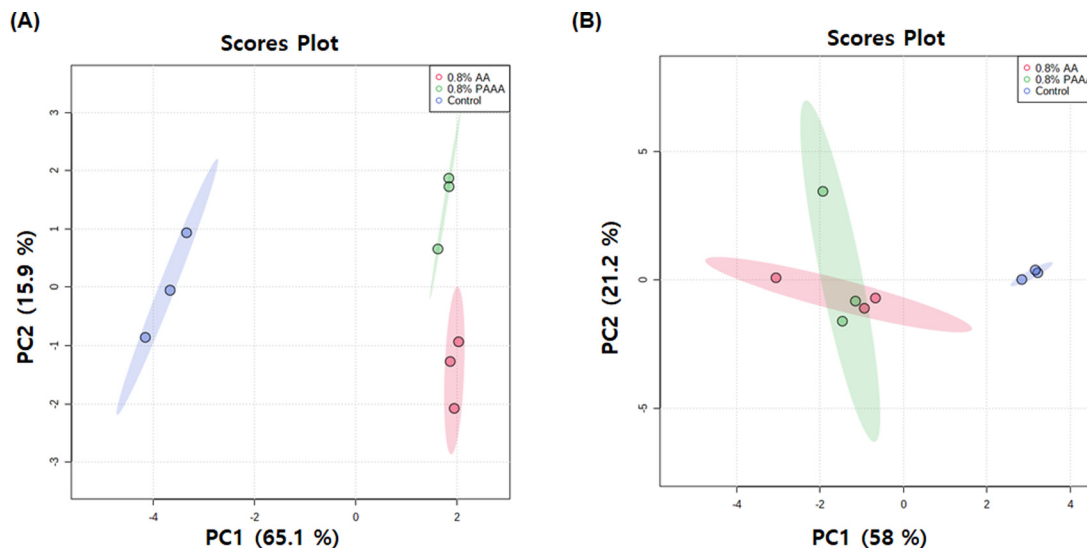
Figure 4. Principal component analysis (PCA) score plots for all samples in different type of treatments. (A) Chicken breasts; (B) drumsticks. Control, deionized water treatment; 0.8% acetic acid (AA), 0.8% AA treatment; 0.8% plasma-activated acetic acid (PAAA), 0.8% PAAA treatment.

in Figure 4. The two principal components (**PC**), PC1 and PC2, of chicken breasts and drumsticks well explained 81 and 79.2% of the overall variance, respectively. PCA is a simple method used for modeling and visualization of multidimensional data (Wiśniewska et al., 2016). PC1 was the most important variable for the different treatments (control, 0.8% AA, and 0.8% PAAA). As for chicken breasts presented in Figure 4A, PC1 separated control from 0.8% AA and 0.8% PAAA and accounted for 65.1% of the total variation, which indicated that there was a significant difference in odors between the control and the treatment groups. However, the difference between 0.8% AA and PAAA was not identified in the PC1 data area, indicating that they were not significantly different in odors. These results were equal to those of the drumsticks presented in Figure 4B. The overlapping area between 0.8% AA and PAAA in the results of drumsticks indicates that there were similarities of odor between 0.8% AA and PAAA treatments. Thus, the significant differences in odors between the control and treatment groups were mainly related to the action of AA, indicating that the changes in odor caused by the plasma treatment were negligible. Previous study found that 2.0% lactic acid treatment changed the odors of beef compared with 0.05 to 0.20% plasma activated lactic acid treatments (Qian et al., 2019).

In the present study, we identified the synergistic antibacterial activity of PAAA and its quality traits in chicken meat. PAAA showed a higher bactericidal efficiency than AA in breasts, drumsticks, and skin, and the bactericidal activity was proportional to the concentration of AA. Based on the 0.8% concentration of AA, which had a high bactericidal activity, there were no significant differences in the quality traits of chicken, including pH, surface color, and TBARS values between AA and PAAA treatments. Similar results were obtained for the morphological images and volatile

compound areas analyzed by SEM and electronic nose analysis, respectively.

In conclusion, PAAA may be a potential decontaminating agent that can reduce foodborne pathogens such as *S.* Typhimurium on chicken meats. Considering the results of this study, PAAA can be a potentially useful agent in the industry to produce safer chicken meat products from the beginning. However, further studies are needed to evaluate the physicochemical, microbiological, and sensory properties of chicken meat after treatment with PAAA during storage and to assess mechanism of antibacterial effect against pathogenic bacteria including *S.* Typhimurium. In addition, the maximum volume of PAAA is limited in the present study, it is valuable to develop different treatment methods of PAAA other than immersion could be useful for industrial application.

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DISCLOSURES

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

REFERENCES

Aktaş, N., M. I. Aksu, and M. Kaya. 2003. The effect of organic acid marination on tenderness, cooking loss and bound water content of beef. J. Muscle Foods 14:181–194.

Alagöz, E., K. Ünal, A. Cabi, and C. Sarıçoban. 2020. Determination of the effect of some acidic solutions on the tenderness and quality properties of chicken breast meat. Selcuk J. Agr. Food Sci. 34:19–23.

An, J. Y., H. I. Yong, H. J. Kim, J. Y. Park, S. H. Lee, K. H. Baek, W. Choe, and C. Jo. 2019. Mechanism of inactivation of Escherichia coli O157:H7 biofilm by different plasma treated solutions and post treatment storage. Appl. Phy. Lett. 114:073703.

Babaeva, N. Y., N. Ning, D. B. Graves, and M. J. Kushner. 2012. Ion activation energy delivered to wounds by atmospheric pressure dielectric-barrier discharges: sputtering of lipid-like surfaces. J. Phys. D. 45:115203.

Bell, M. F., R. T. Marshall, and M. E. Anderson. 1986. Microbiological and sensory tests of beef treated with acetic and formic acids. J. Food Prot. 49:207–210.

Berrang, M. E., J. S. Bailey, S. F. Altekruse, B. Patel, W. K. Shaw Jr, R. J. Meinersmann, and P. J. Fedorka-Cray. 2007. Prevalence and numbers of Campylobacter on broiler carcasses collected at rehang and postchill in 20 US processing plants. J. Food Prot. 70:1556–1560.

Berri, C., N. Wacrenier, N. Millet, and E. Le Bihan-Duval. 2001. Effect of selection for improved body composition on muscle and meat characteristics of broilers from experimental and commercial lines. Poult. Sci. 80:833–838.

Chen, Q., Y. Hu, R. Wen, Y. Wang, L. Qin, and B. Kong. 2021. Characterisation of the flavour profile of dry fermented sausages with different NaCl substitutes using HS-SPME-GC-MS combined with electronic nose and electronic tongue. Meat Sci. 172:108338.

Cosansu, S., and K. Ayhan. 2010. Effects of lactic and acetic acid treatments on Campylobacter jejuni inoculated onto chicken leg and breast meat during storage at 4°C and -18°C. J. Food Process. Preserv. 34:98–113.

Denicola, A., J. M. Souza, and R. Radi. 1998. Diffusion of peroxynitrite across erythrocyte membranes. Proc. Natl. Acad. Sci. 95:3566–3571.

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

Domínguez, C., I. Gomez, and J. Zumalacarregui. 2002. Prevalence of Salmonella and Campylobacter in retail chicken meat in Spain. Int. J. Food Microbiol. 72:165–168.

Fröhling, A., J. Durek, U. Schnabel, J. Ehlbeck, J. Bolling, and O. Schlüter. 2012. Indirect plasma treatment of fresh pork: decontamination efficiency and effects on quality attributes. Innov. Food Sci. Emerg. Technol. 16:381–390.

Hamilton, M. (Ed.). (2003). Cytergy, Univ. Montana State, Bozeman, Montana.

Heo, Y. S., D. G. Yim, K. H. Baek, T. Kang, Y. E. Lee, J. Kim, W. Choe, and C. Jo. 2021. Effect of inkjet-printed flexible dielectric barrier discharge plasma on reduction of pathogen and quality changes on sliced cheese. LWT 143:111128.

Huie, R. E., and S. Padmaja. 1993. The reaction of NO with superoxide. Free Radic. Res. Commun 18:195–199.

Jayasena, D. D., H. J. Kim, H. I. Yong, S. Park, K. Kim, W. Choe, and C. Jo. 2015. Flexible thin-layer dielectric barrier discharge plasma treatment of pork butt and beef loin: effects on pathogen inactivation and meat-quality attributes. Food Microbiol 46:51–57.

Kang, S. N., A. Jang, S. O. Lee, J. S. Min, and M. Lee. 2002. Effect of organic acid on value of VBN, TBARS, color and sensory property of pork meat. J. Anim. Sci. Technol. 44:443–452.

Kang, C., Q. Xiang, D. Zhao, W. Wang, L. Niu, and Y. Bai. 2019. Inactivation of Pseudomonas deceptionensis CM2 on chicken breasts using plasma-activated water. J Food Sci Technol 56:4938–4945.

Kim, H. C., K. H. Baek, Y. J. Ko, H. J. Lee, D. G. Yim, and C. Jo. 2020. Characteristic metabolic changes of the crust from dry-aged beef using 2D NMR spectroscopy. Molecules 25:3087.

Kim, H. J., J. Sujiwo, H. J. Kim, and A. Jang. 2019. Effects of dipping chicken breast meat inoculated with Listeria monocytogenes in lyophilized scallion, garlic, and kiwi extracts on its physicochemical quality. Food Sci. Anim. Resour. 39:418–429.

Kim, T., J. L. Silva, and T. C. Chen. 2002. Effects of UV irradiation on selected pathogens in peptone water and on stainless steel and chicken meat. J. Food Protect. 65:1142–1145.

Laurita, R., D. Barbieri, M. Gherardi, V. Colombo, and P. Lukes. 2015. Chemical analysis of reactive species and antimicrobial activity of water treated by nanosecond pulsed DBD air plasma. Clin. Plasma Med 3:53–61.

Lee, D., H. J. Lee, J. W. Yoon, M. Kim, and C. Jo. 2021a. Effect of different aging methods on the formation of aroma volatiles in beef strip loins. Foods 10:146.

Lee, H. J., J. Choe, M. Kim, H. C. Kim, J. W. Yoon, S. W. Oh, and C. Jo. 2019. Role of moisture evaporation in the taste attributes of dry-and wet-aged beef determined by chemical and electronic tongue analyses. Meat Sci 151:82–88.

Lee, H., H. I. Yong, H. J. Kim, W. Choe, S. J. Yoo, E. J. Jang, and C. Jo. 2016. Evaluation of the microbiological safety, quality changes, and genotoxicity of chicken breast treated with flexible thin-layer dielectric barrier discharge plasma. Food Sci. Biotechnol. 25:1189–1195.

Lee, J., J. M. Sung, H. J. Cho, S. H. Woo, M. C. Kang, H. I. Yong, T. K. Kim, H. Lee, and Y. S. Choi. 2021. Natural extracts as inhibitors of microorganisms and lipid oxidation in emulsion sausage during storage. Food Sci. Anim. Resour 41:1060–1077.

Li, X., I. Amadou, G. Y. Zhou, L. Y. Qian, J. L. Zhang, D. L. Wang, and X. R. Cheng. 2020. Flavor components comparison between the neck meat of donkey, swine, bovine, and sheep. Food Sci. Anim. Resour. 40:527–540.

Lin, C. M., Y. C. Chu, C. P. Hsiao, J. S. Wu, C. W. Hsieh, and C. Y. Hou. 2019. The optimization of plasma-activated water treatments to inactivate Salmonella Enteritidis (ATCC 13076) on shell eggs. Foods. 8:520.

Liu, F., P. Sun, N. Bai, Y. Tian, H. Zhou, S. Wei, Y. Zhou, J. Zhang, W. Zhu, K. Becker, and J. Fang. 2010. Inactivation of bacteria in an aqueous environment by a direct-current, cold-atmospheric-pressure air plasma microjet. Plasma Process. Polym. 7:231–236.

Lukes, P., E. Dolezalova, I. Sisrova, and M. Clupek. 2014. Aqueous-phase chemistry and bactericidal effects from an air discharge plasma in contact with water: evidence for the formation of peroxynitrite through a pseudo-second-order post-discharge reaction of H_2O_2 and HNO_2. Plasma Sources Sci. Technol. 23:015019.

Mani-López, E., H. S. García, and A. López-Malo. 2012. Organic acids as antimicrobials to control Salmonella in meat and poultry products. Food Res. Int. 45:713–721.

Naïtali, M., G. Kamgang-Youbi, J. M. Herry, M. N. Bellon-Fontaine, and J. L. Brisset. 2010. Combined effects of long-living chemical species during microbial inactivation using atmospheric plasma-treated water. Appl. Environ. Microbiol. 76:7662–7664.

Nerin, C., M. Aznar, and D. Carrizo. 2016. Food contamination during food process. Trends Food Sci. Technol. 48:63–68.

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

Oehmigen, K., M. Hähnel, R. Brandenburg, C. Wilke, K. D. Weltmann, and T. Von Woedtke. 2010. The role of acidification for antimicrobial activity of atmospheric pressure plasma in liquids. Plasma Process. Polym. 7:250–257.

Oehmigen, K., J. Winter, M. Hähnel, C. Wilke, R. Brandenburg, K. D. Weltmann, and T. von Woedtke. 2011. Estimation of possible mechanisms of Escherichia coli inactivation by plasma treated sodium chloride solution. Plasma Process. Polym. 8:904–913.

Omer, M. K., A. Alvarez-Ordonez, M. Prieto, E. Skjerve, T. Asehun, and O. A. Alveseike. 2018. A systematic review of bacterial foodborne outbreaks related to red meat and meat products. Foodborne Pathog. Dis. 15:598–611.

Qian, J., C. Wang, H. Zhuang, J. Zhang, and W. Yan. 2020. Oxidative stress responses of pathogen bacteria in poultry to plasma-activated lactic acid solutions. Food Control 118:107355.

Qian, J., H. Zhuang, M. M. Nasiru, U. Muhammad, J. Zhang, and W. Yan. 2019. Action of plasma-activated lactic acid on the inactivation of inoculated Salmonella Enteritidis and quality of beef. Innov. Food Sci. Emerg. Technol. 57:102196.

Reis, J. A., A. T. Paula, and S. N. Casarotti. 2012. Lactic acid bacteria antimicrobial compounds: characteristics and applications. Food Eng. Rev. 4:124–140.

Rossow, M., M. Ludewig, and P. G. Braun. 2018. Effect of cold atmospheric pressure plasma treatment on inactivation of *Campylobacter jejuni* on chicken skin and breast fillet. LWT 91:265–270.

Royintarat, T., E. H. Choi, D. Boonyawan, P. Seesuriyachan, and W. Wattanutchariya. 2020. Chemical-free and synergistic interaction of ultrasound combined with plasma-activated water (PAW) to enhance microbial inactivation in chicken meat and skin. Sci. Rep. 10:1–14.

Sagong, H. G., S. Y. Lee, P. S. Chang, S. Heu, S. Ryu, Y. J. Choi, and D. H. Kang. 2011. Combined effect of ultrasound and organic acids to reduce *Escherichia coli* O157: H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* on organic fresh lettuce. Int. J. Food Microbiol. 145:287–292.

Samukawa, S., M. Hori, S. Rauf, K. Tachibana, P. Bruggeman, G. Kroesen, J. C. Whitehead, A. B. Murphy, A. F. Gutsol, S. Starikovskaia, U. Kortshagen, J. Boeuf, T. J. Sommerer, M. J. Kushner, U. Czarnetzki, and N. Mason. 2012. The 2012 plasma roadmap. J. Phys. D. 45:253001.

Shin, D. J., H. J. Lee, D. Lee, C. Jo, and J. Choe. 2020. Fat replacement in chicken sausages manufactured with broiler and old laying hens by different vegetable oils. Poult. Sci. 99:2811–2818.

Stivarius, M. R., F. W. Pohlman, K. S. McElyea, and J. K. Apple. 2002. The effects of acetic acid, gluconic acid and trisodium citrate treatment of beef trimmings on microbial, color and odor characteristics of ground beef through simulated retail display. Meat Sci. 60:245–252.

Surekha, M., and S. M. Reddy. 2000. Preservatives, classification and properties. Pages 1710−1717 in Encyclopedia of Food Microbiology. R. K. Robinson, C. A. Batt and C. Patel, eds. Academic Press, New York, NY.

Von Lengerken, G., S. Maak, and M. Wicke. 2002. Muscle metabolism and meat quality of pigs and poultry. Vet. Zootech. 20:82–86.

Wadhwani, R., and D. J. McMahon. 2012. Color of low-fat cheese influences flavor perception and consumer liking. J. Dairy Sci. 95:2336–2346.

Whyte, P., J. D. Collins, K. McGill, C. Monahan, and H. O'mahony. 2001. Quantitative investigation of the effects of chemical decontamination procedures on the microbiological status of broiler carcasses during processing. J. Food Prot. 64:179–183.

Wiśniewska, P., M. Śliwińska, J. Namieśnik, W. Wardencki, and T. Dymerski. 2016. The verification of the usefulness of electronic nose based on ultra-fast gas chromatography and four different chemometric methods for rapid analysis of spirit beverages. J. Anal. Methods Chem. 2016:8763436.

Yim, D. G., D. J. Shin, C. Jo, and K. C. Nam. 2020. Effect of sodium-alternative curing salts on physicochemical properties during salami manufacture. Food Sci. Anim. Resour. 40:946–956.

Yong, H. I., S. H. Lee, S. Y. Kim, S. Park, J. Park, W. Choe, and C. Jo. 2019. Color development, physiochemical properties, and microbiological safety of pork jerky processed with atmospheric pressure plasma. Innov. Food Sci. Emerg. Technol. 53:78–84.

Yoo, J. H., J. W. Kim, H. I. Yong, K. H. Baek, H. J. Lee, and C. Jo. 2020. Effects of searing cooking on sensory and physicochemical properties of beef steak. Food Sci. Anim. Resour. 40:44–54.

Yoo, J. H., K. H. Baek, Y. S. Heo, H. I. Yong, and C. Jo. 2021. Synergistic bactericidal effect of clove oil and encapsulated atmospheric pressure plasma against *Escherichia coli* O157: H7 and *Staphylococcus aureus* and its mechanism of action. Food Microbiol. 93:103611.

Zhao, C., B. Ge, J. De Villena, R. Sudler, E. Yeh, S. Zhao, D. G. White, D. Wagner, and J. Meng. 2001. Prevalence of *Campylobacter* spp., *Escherichia coli*, and *Salmonella* serovars in retail chicken, turkey, pork, and beef from the Greater Washington, DC, area. Appl. Environ. Microbiol. 67:5431–5436.

Zhou, R., X. Zhang, Z. Bi, Z. Zong, J. Niu, Y. Song, D. Liu, and S. Yang. 2015. Inactivation of *Escherichia coli* cells in aqueous solution by atmospheric-pressure N2, He, Air, and O2 microplasmas. Appl. Environ. Microbiol. 81:5257–5265.