A Dissertation for the Degree of Master

# Bactericidal effects and quality characteristics of pork and chicken meat treated by plasma-activated organic acids

돈육 및 계육에 대한 플라즈마 활성 유기산의 살균 효과 및 품질 특성 확인

August 2023

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# Abstract

# Bactericidal effects and quality characteristics of pork and chicken meat treated by plasma-activated organic acids

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Plasma-activated organic acid (PAOA), which is produced by combined treatment of plasma and organic acid, was used to increase the bactericidal effect on pork and chicken and to improve their oxidative stability. PAOA was produced by treating the organic acid surface with plasma discharge, and the antibacterial effect, physicochemical quality, and oxidation stability of pork and chicken meat immersed in various PAOA were confirmed. The purpose of this study is to investigate whether the use of PAOA can be a technology that can improve the safety and oxidative stability of meat.

**Experiment I.**

**Effect of plasma-activated organic acids against *Salmonella* Typhimurium and *Escherichia coli* O157:H7 inoculated on pork loin and its quality characteristics**

This study investigated the effectiveness of plasma-activated organic acid (PAOA) on the bactericidal effect and quality characteristics of pork loin. Three different pathogens were used to inoculate the pork loins, and the results showed that PAOA exhibited a higher reduction level than plasma-activated water, which can be attributed to its lower pH, higher oxidation-reduction potential, and reactive oxygen species concentrations. Among the PAOA treatments, 0.5% plasma-activated acetic acid (PAA) showed a synergistic bactericidal effect against *Salmonella* Typhimurium and *Escherichia coli* O157:H7 compared to untreated organic acid. PAA also outperformed other PAOA treatments in terms of pork quality with similar meat color to deionized water, no adverse effect on lipid oxidation, and significantly reduced protein oxidation. Sensory analysis identified PAA as having the highest umami taste. Thus, PAA is a promising method to control microbial contamination in the meat industry while enhancing the oxidative stability and umami taste of pork loin.

**Experiment II.**

**Synergistic effects of plasma and organic acids on bactericidal effect and antioxidant activity of chicken breast and drumstick**

Lactic acid, gallic acid, and their mixture (1% each) were prepared (LA, GA, and LGA) and plasma-activated organic acids (PAOA) were produced through exposure of 1% organic acid to plasma for 1 h (PAL, PAG, and PLGA). Chicken breast and drumstick were immersed in the prepared solutions for 10 min and analyzed their antibacterial effect against *Salmonella* Typhimurium and *Campylobacter jejuni* and antioxidant activity during 12 days of storage. As a result, PAOA inactivated approximately 6.37 Log CFU/mL against *S*. Typhimurium and 2.76, 1.86, and 3.04 Log CFU/mL against *C. jejuni* (PAL, PAG, and PLGA, respectively). Moreover, PAOA had bactericidal effect in both chicken parts inoculated with pathogens, with PAL and PLGA displaying higher antibacterial activity compared to PAG. Meanwhile, PAOA inhibited lipid oxidation in chicken meats, and PAG and PLGA had higher oxidative stability during storage compared to PAL. This can be attributed to the superior antioxidant properties of GA and LGA, including higher total phenolic contents, $ABTS^{+}$ reducing activity, and DPPH radical scavenging activity, when compared to LA. In particular, when combined with plasma treatment, LGA showed the greatest improvement in antioxidant activity compared to other organic acids. In summary, PLGA not

only had a synergistic bactericidal effect against pathogens on chicken, but also improved oxidative stability during storage. Therefore, PLGA can be an effective method for controlling microorganisms without adverse effect on lipid oxidation for different chicken cuts.

**Keywords**: Plasma-activated organic acids, Pork loin, Chicken meats, Pathogens, Antibacterial effect, Physicochemical quality, Antioxidant activity

**Student Number**: 2021-28336

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# List of Abbreviations

| | | |
|---|---|---|
| *a** | : | Redness |
| AA | : | Acetic acid |
| $ABTS^+$ | : | 2, 2'-azinobis (3-ethylbenzothiazol ine-6-sulfonic acid) radical cation |
| *b** | : | Yellowness |
| CA | : | Citric acid |
| CFU | : | Colony-forming unit |
| DBD | : | Dielectric barrier discharge |
| DDW | : | Deionized water |
| DPPH | : | 2, 2-diphenyl-1-picryl-hydrazyl-hydrate |
| GA | : | Gallic acid |
| H | : | Hours |
| $H_2O_2$ | : | Hydrogen peroxide |
| IMP | : | Inosine 5′-monophosphate |
| *L** | : | Lightness |
| LA | : | Lactic acid |
| LGA | : | Mixed of lactic acid and gallic acid |
| MDA | : | Malondialdehyde |
| Min | : | Minutes |

| | | |
|---|---|---|
| ND | : | Not detected |
| $O_3$ | : | Ozone |
| ORP | | Oxidation-reduction potential |
| PAA | : | Plasma-activated acetic acid |
| PAC | : | Plasma-activated citric acid |
| PAG | : | Plasma-activated gallic acid |
| PAL | : | Plasma-activated lactic acid |
| PAOA | : | Plasma-activated organic acid |
| PAW | : | Plasma-activated water |
| PCA | : | Principal component analysis |
| PLGA | : | Plasma-activated lactic-gallic acid |
| ROS | : | Reactive oxygen species |
| TBARS | : | 2-thiobarbituric acid reactive substance |
| Trolox | : | 6-hydroxy-2,5,7,8 tetramethylchroman-2-carboxylic acid |

# Chapter I.

# General introduction

Consumer awareness of the safety of meat and meat products is increasing along with the increase in meat consumption (Baek et al., 2020; Nerin et al., 2016). Meat has rich nutrient composition, which is a good environment for microbial growth and is susceptible to contamination by microorganisms (Yoo et al., 2021. Pathogens that can cause contamination in meat include *Salmonella* Typhimurium, *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Campylobacter jejuni* (Hatanaka et al., 2020; Kang et al., 2022a), which may cause food poisoning that can cause abdominal pain, vomiting, diarrhea, and high fever (Gourama, 2020). Therefore, for the safe consumption of meat, a technology that can efficiently control microorganisms in meat and meat product is required.

Plasma is one of the non-thermal technologies and means ionized gas under quasi-neutral conditions (Lee et al., 2011). plasma consists of ions, electrons, Ultraviolet photons, and neutral particles including free radicals and reactive species (Qian et al., 2021). In particular, reactive oxygen species such as hydroxyl radical, hydrogen peroxide, superoxide anion, and ozone generated during plasma discharge can induce oxidative stress on the cell membrane and intracellular materials of bacterial cells, effectively inactivating microorganisms (Yong et al. al., 2015). Compared to other non-thermal technologies, plasma has the advantage of being

easy to operate, low cost, and an environmentally friendly technology that leaves no residue after treatment (Qian et al., 2021a). However, direct treatment using gas plasma has limitations in that it has a low penetration depth into meat and may cause excessive oxidative stress, which can deteriorate the nutritional and sensory properties of meat. (Domonkos et al., 2121; Jayasena et al., 2015).

PAW is produced by gas plasma treatment on the surface of water and contains various reactive species (Gao et al 2022). PAW offers efficient inhibition of microorganism growth through the utilization of reactive species, while exerting a lesser impact on the nutritional and sensory quality of meat compared to gas plasma treatment (Zhou e al., 2020). Furthermore, PAW can be produced on a large scale and can be applied to food in various forms, providing additional advantages (Gao et al., 2022; Royintarat et al., 2020). However, PAW has a limitation in reducing its bactericidal effect against organic matter, and some studies have reported that PAW can still cause excessive oxidation in meat (Baek et al., 2020; Kim et al., 2013). Therefore, it is necessary to develop methods that can enhance the antibacterial effect and antioxidant activity of PAW to efficiently control microorganisms present in meat without adversely affecting oxidative stability.

Organic acids are widely recognized as effective antibacterial agents in the food industry and have demonstrated their ability to efficiently inactivate microorganisms (Zhou et al., 2023). Furthermore, certain organic acids exhibit not only antibacterial properties but also exceptional antioxidant activity (Zahrani et al., 2020). Therefore, when plasma is combined with organic acids, various reactive species generated by plasma treatment can be dissolved in organic acids to improve antibacterial effects,

and the antioxidant activity of organic acids may address excessive oxidation stress caused by PAW (Kang et al., 2022b; Qian et al., 2019). This study aims to overcome the limitations of PAW, including its limited antibacterial activity and excessive oxidative stress on organic matter, through the combined treatment of plasma and various organic acids.

# Chapter II.

# Effect of plasma-activated organic acids against *Salmonella* Typhimurium and *Escherichia coli* O157:H7 inoculated on pork loin and its quality characteristics

This manuscript consists of part of a paper submitted to Innovative Food Science & Emerging Technologies as partial fulfillment of the Master's program of Hag Ju Lee.

## 2.1. Introduction

The global increase in meat consumption has led to a growing concern for the quality and safety of meat and meat products among consumers. Over the past two decades, there has been a significant improvement in consumers' perception of meat safety (Nerin et al., 2016). The main cause of food poisoning is still contamination by pathogenic bacteria such as *Salmonella* spp, *Escherichia coli*, and *Listeria monocytogenes* (Kang et al., 2022a), which can occur during various stages of meat

production, processing, packaging, and distribution (Yoo et al., 2021). Thermal technologies are a representative method for preventing contamination by microorganisms (Huang et al., 2020), but they can adversely affect the nutritional and sensory quality of meat (Liao et al., 2017). To overcome this issue, various non-thermal technologies such as ultrasound, irradiation, high pressure, and plasma are emerging (Osae et al., 2020). Among these, cold plasma, composed of charged parts, ultraviolet photons, atoms, and free radicals (Baek et al., 2021), has the advantage of efficiently inactivating microorganisms with no residue left after treatment and relatively easy operation (Qian et al., 2021a). However, direct treatment with gas plasma may cause excessive oxidation of the meat and deteriorate its physicochemical properties (Qian et al., 2021a). To address this problem, some studies have suggested using PAW, an indirect plasma treatment method that might minimize changes in food characteristics (Royintarat et al., 2020; Xiang et al., 2022). PAW, which is generated by subjecting water to atmospheric plasma discharge, contains a variety of reactive species that can significantly contribute to its antibacterial effect (Gao et al., 2022). However, PAW has a limitation in its bactericidal effect when treated that contains organic matter, such as food (Baek et al., 2020).

To enhance the bactericidal effect of PAW for meat, some studies have attempted to improve it through the combined treatment of plasma and organic acid (Qian et al., 2020). Previously, there are studies reported with plasma-acetic acid combination for chicken meat and plasma-lactic acid combination for chicken meat and beef, respectively (Qian et al., 2019; Qian et al., 2021b; Kang et al., 2022b; Zhao et al.,

2021). The aforementioned studies on PAOA have consistently demonstrated a greater bactericidal effect when applied to food samples compared to individual treatments of plasma or organic acid alone. However, most experiments were conducted mainly on one type of bacterial strain for antibacterial activity using chicken meat and beef, and the bactericidal effects of various types of PAOA were not compared.

Thus, the aim of the present study was to confirm the bactericidal effect of PAOA including acetic acid, lactic acid, and citric acid, on pork loin inoculated with *S.* Typhimurium, *E. coli* O157:H7, and *L. monocytogenes*, respectively. Moreover, the physicochemical properties of plasma-activated solution and the quality properties of pork loin treated with PAOA were also evaluated.

## 2.2. Materials and methods

### *2.2.1. Bacterial solution and sample preparation*

In this study, three pathogenic bacteria, *S.* Typhimurium (ATCC 14411), *E. coli* O157:H7 (NCCP 15739), and *L. monocytogenes* (ATCC 19111), were utilized. The bacteria were obtained from American Type Culture Collection (Virginia, USA), National Culture Collection for Pathogens (Osong, Korea), and Korean Culture Center of Microorganisms (Seoul, Korea), respectively. *S.* Typhimurium, *E.* coli O157:H7, and *L.* monocytogenes were cultivated on nutrient agar, tryptic soy agar, and TSA containing yeast extract, respectively. The single colony of each pathogenic bacteria was transferred to 25 mL nutrient broth, tryptic soy broth, and tryptic soy broth containing yeast extract, respectively. The bacterial strains were cultured in a broth for 24 hours at 37°C with orbital agitation set at 120 rpm. Subsequently, the cultures were transferred to fresh broth and further cultured for 18 hours under the same temperature and agitation conditions. After incubation, each broth was transferred to a 50 mL centrifuge tube and washed twice using a centrifuge at 4,001 ×g and 4°C for 10 min. After centrifugation, the supernatant was discarded, 0.85% NaCl was added and diluted to the cell pellet, and each bacterial solution was adjusted to a concentration of about $10^6$-$10^7$ log CFU/mL before use. The final concentration of the bacterial solution was confirmed by measuring the optical density at 600 nm ($OD_{600}$ = 0.2).

In this experiment, pork loin samples were obtained from a domestic market (Seoul, Korea) at 24 h postmortem. Before sample preparation, microorganisms

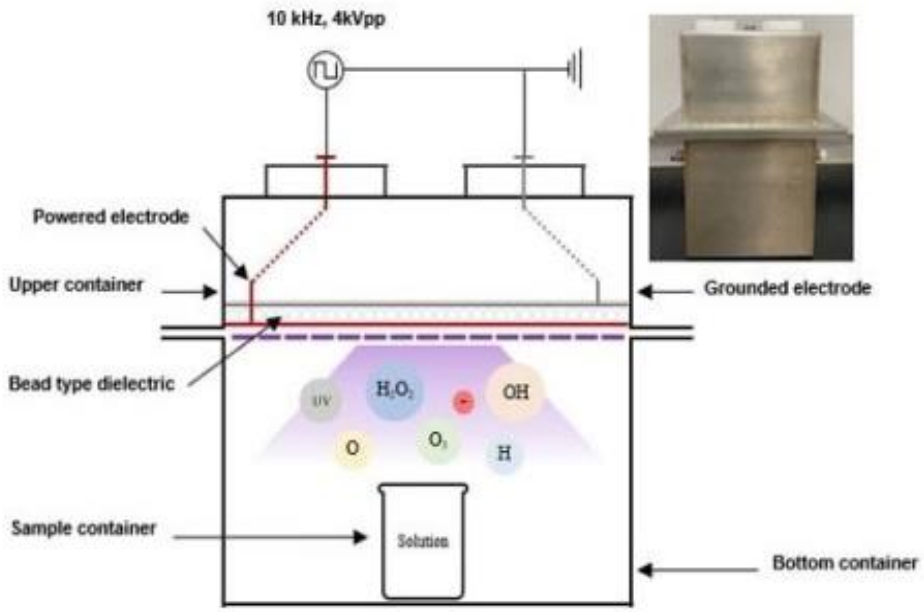
present on the pork loin surface were removed using a food sterilization disinfectant (Jinro-Distillers, Ansan, Gyeonggi, Korea) and ultraviolet light. Subsequently, the pork loin was cut into pieces of 5 g using sterilized forceps and knives and then used in the experiment.

### *2.2.2. Preparation of plasma*-activated *solution*

Figure. 1 shows a schematic diagram of the dielectric barrier discharge plasma system used in this study. The container of the plasma device was made of zirconium material, and the size of the bottom container containing the sample was 15 × 15 × 15 cm. The upper container was 15 × 15 × 9 cm, and the plasma generator was attached to the bottom (Figure. 1A). The size of the plasma generator was 15 × 15 × 1 cm, a bead-type dielectric was used, and grounded electrodes and powered electrodes were attached above and below the dielectric (Figure. 1B). Plasma discharge was performed under conditions of 10 kHz and 4.0 kVpp using atmospheric air.

In this experiment, PAW, 0.5% and 1.0% PAA, PAL, and PAC were used. The determined concentration of PAOA was set through preliminary experiments. The plasma-activated solution was produced by discharging DBD plasma to 200 mL of DDW, 0.5% and 1.0% AA, LA, and CA, respectively. Plasma was treated at 12 cm above the solution for 60 min, and the solution was stirred at 120 rpm using a stirrer during discharging.

**(A)**




**(B)**

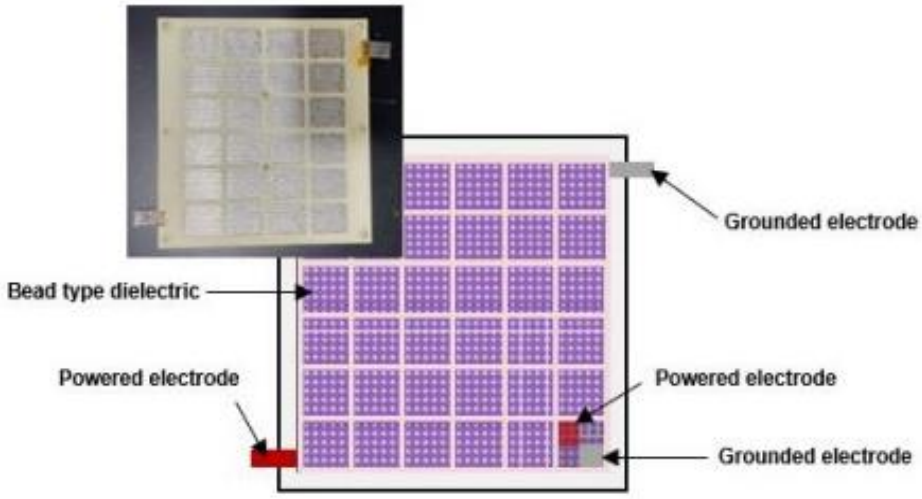



Figure 1. Schematic diagram of (A) the Bead-type DBD plasma system and (B) gas plasma generator used to prepare plasma-activated solution.

### *2.2.3. Microbial analysis*

To confirm the bactericidal effect of PAW and PAOA on *S*. Typhimurium, *E. coli* O157:H7, *L*. monocytogenes, and inoculated organic matter, experiments were conducted by dividing the experimental subjects into bacterial solution and inoculated pork loin.

#### *2.2.3.1. Bactericidal effect on bacterial solution*

After adding 9.9 mL of the treated solution to a centrifuge tube containing 100 μL of each bacterial solution, the mixture was vortexed for 10 sec and allowed to react for 10 min. The mixed solution in the centrifuge tube was diluted to decimal serial dilution according to the appropriate dilution multiplier using sterilized 0.5% NaCl. A hundred microliters of the diluted solution were spread on Xylose Lysine Deoxycholate agar (*S*. Typhimurium selective agar), Eosin Methylene Blue agar (*E. coli* O157:H7 selective agar), Listeria Selective agar (*L. monocytogenes* selective agar) agar. After incubated at 37°C for 48 h, the number of colonies was counted and expressed as log CFU/mL.

#### *2.2.3.2. Bactericidal effect on inoculated pork loin*

After spot inoculation of 100 μL of bacterial solution on the surface of a 5 g pork loin piece, it was dried at room temperature (25 ± 2°C) for 30 min. After immersing the inoculated sample in each treatment solution for 10 min, the moisture on the sample's surface was removed, and it was then transferred to a sterile bag

containing 45 mL of sterile 0.85% NaCl. Then, it was homogenized using a stomacher for 2 min to detach the bacterial strain presented in the sample. The homogenized solution was diluted to decimal serial dilution according to the appropriate dilution multiplier using sterilized 0.5% NaCl. Finally, 100 μL of the diluted solution was spread on Xylose Lysine Deoxycholate agar, Eosin Methylene Blue agar, and Listeria Selective agar, and after incubation at 37°C for 48 h, the number of colonies was counted and expressed as log CFU/g.

### *2.2.4. Physicochemical properties of plasma*-activated *solution*

#### *2.2.4.1. pH and ORP measurement*

The pH and ORP values of the untreated and plasma-activated solutions were measured immediately after generation using a pH meter (Seven 2Go, Mettler-Toledo International Inc., Schwerzenbach, Switzerland) and an ORP sensor (ORP electrode InLab Redox, Mettler Toledo, Schwerzenbach, Switzerland).

#### *2.2.4.2. ROS measurement*

The concentration of hydrogen peroxide dissolved in the solution was analyzed according to the method of park et al. (2017) with some modifications. Add 1 mL of 10 mM ammonium metavanadate (Sigma-Aldrich, St. Louis, MO, USA) and 0.3 mL of 5 M sulfuric acid (Duksan Pure Chem, Ansan, Gyeonggi, Korea) to a centrifuge tube containing 1 mL of the sample (untreated or plasma-activated solution), and

then reacted it for 2 min. Then, after transferring the mixed sample to a cuvette, absorbance was measured at 450 nm using a spectrophotometer (X-ma 3100, Human Co Ltd., Seoul, Korea). The measured absorbance was analyzed using a standard curve to calculate the hydrogen peroxide concentration.

The concentration of ozone dissolved in the solution was analyzed according to the method of the Greenberg et al. (1992) with some modifications. 20 mM phosphoric acid (Duksan Pure Chem, Ansan, Gyeonggi, Korea) and 1 mM potassium indigo trisulfonate (Sigma-Aldrich, St. Louis, MO, USA) were dissolved in DDW to make an indigo stock. Then, dissolve 20 mL indigo stock solution, 10 g sodium dihydrogen phosphate (Sigma-Aldrich, St. Louis, MO, USA), and 7 mL phosphoric acid (20 mM) in DDW to make indigo reagent I. In a 100 mL volumetric flask, add 10 mL of indigo stock solution and 90 mL of a sample (untreated or plasma-activated solution) and shake it so that it does not create bubbles to remove the decolorized zone. After transferring each solution to a cuvette, absorbance was measured at 600 nm using a spectrophotometer (X-ma 3100, Human Co Ltd). Calculate the ozone concentration using the equation below.

$$\text{mg } O_3/L = \frac{100 \times \Delta A}{f \times b \times V}$$

$\triangle A$: absorbance difference between sample and blank, $b$: width of cuvette (cm), $V$: amount of sample (typically 90 mL), f: 0.42

### *2.2.5. Physicochemical properties of pork loin*

The physicochemical properties of the pork loin were confirmed not only

immediately after treatment with the treated solution but also after refrigerated storage at 4 ± 0.2°C for 3 and 6 days.

### *2.2.5.1. pH and surface color measurement*

After adding 9 mL of DDW to the centrifuge tube containing the minced 1 g sample, homogenized using a homogenizer (T25 Basic, Ika Co., Staufen, Germany) for 30 s. The homogenate sample was centrifuged at 2,265 ×g, 10 min, 4°C, and the supernatant was filtered, and the pH value was measured using a pH meter (Seven 2Go, Mettler-Toledo International Inc).

The color of the pork loin immersed in the treated solution for 10 min was measured using a colorimeter (CM-5, Konica Minolta Co., Ltd., Osaka, Japan). Calibration of the instrument before the measurement was conducted through black and white tiles, and measurement was performed with an 8 mm diameter using illuminant D65 (Lee et al., 2022). The color of the sample was expressed through CIE color $L^*$ (darkness-brightness), $a^*$ (greenness-redness), and $b^*$ (blueness-yellowness).

### *2.2.5.2. Lipid and protein oxidation measurement*

The TBARS value was used to determine the lipid oxidation and was carried out with slight modifications from the method described by Jung et al. (2022). Add 15 mL of DDW and 50 μL of 7.2% butylated hydroxyl toluene (Sigma-Aldrich, St. Louis, MO, USA) to a centrifuge tube containing a 5 g of sample and homogenize

for 30 s. After centrifuging the homogenized sample at 2,265 ×g, 15 min, 4°C, the supernatant is filtered. Then, transfer 2 mL of the sample solution to a centrifuge tube and add 4 mL of 20 mM 2-thiobarbituric acid (Sigma-Aldrich, St. Louis, MO, USA) in 15% trichloroacetic acid (Alfa Aesar, Ward Hill, MA, USA). Heat the centrifuge tube in a water bath at 90°C for 30 min, cool it for 15 min, and centrifuge it under the same conditions as above. The absorbance of the supernatant was measured at 532 nm using a spectrophotometer (M2e, Molecular Devices, Sunnyvale, CA), and the TBARS value was expressed as mg MDA/kg of meat sample.

Protein oxidation was analyzed using total carbonyl content, according to the method described by Lee et al. (2021) with some modifications. Meat samples were extracted using 20 mM sodium phosphate. To determine the protein content, the pellet of the extracted sample was treated with 2 M hydrochloric acid and 10% 2-thiobarbituric acid. After centrifugation, the supernatant was discarded, and the pellet was used. After adding 6 M guanidine hydrochloric acid (Duksan Pure Chem, Ansan, Gyeonggi, Korea) in 20 mM sodium phosphate to the pellet, the absorbance was measured at 280 nm using a spectrophotometer (X-ma 3100, Human Co Ltd). The standard curve was made using bovine serum albumin (Sigma-Aldrich, St. Louis, MO, USA). Carbonyl content was measured by adding 0.2% dinitrophenylhydrazine (Duksan Pure Chem, Ansan, Gyeonggi, Korea) to the pellet of the extracted sample, centrifuging, removing the supernatant, and washing with ethanol and ethyl-acetate (1:1, v/v). Then, after adding 6 M guanidine hydrochloric acid in 20 mM sodium phosphate, the absorbance was measured at 370 nm. Carbonyl content was expressed as nmol carbonyls $mg^{-1}$ using a molar absorptivity of 22,000 $M^{-1}cm^{-1}$.

### *2.2.5.3. Electronic tongue profile*

An electronic tongue (Astree, Alpha MOS, Toulouse, France) was used to determine the effects of untreated and plasma-activated solutions on the taste attributes of the sample. Taste attributes were expressed as AHS, CTS, NMS, SCS, and ANS, respectively representing sourness, saltiness, umami, bitterness, and sweetness. Homogenize a 20 g of ground meat sample with 60 mL of DDW. After centrifuging the homogenized sample at 2,265 ×g for 10 min, the supernatant was filtered and stored in a glass bottle under refrigerated conditions (20 ± 2°C) before analysis.

### *2.2.6. NMR-based metabolite analysis*

1D $^{1}$H NMR analysis was performed according to the method described by Kim et al. (2021). Add 20 mL of 0.6 M perchloric acid to a 5 g sample and homogenize. After centrifuging the homogenized sample at 3,000 ×g for 20 min, transfer the supernatant to a new centrifuge tube and adjust the pH to 7.0 using potassium hydroxide. Centrifuge once more under the above conditions, filter, transfer the solution to a new centrifuge tube, and freeze-dry. Dissolve the lyophilized sample in 1 mL deuterium oxide containing 1 mM 3-(trimethylsilyl) propionic-2, 2, 3, 4-$d_4$ acid (TSP) and centrifuge at 3,000 ×g for 5 min. After transferring the supernatant to a microtube centrifuge tube, centrifuging at 17,800 ×g for 20 min, transferring the supernatant to an NMR test tube, and conducting NMR analysis. 1D $^{1}$H NMR spectra

were obtained using a Bruker 850 MHz cryo-NMR spectrometer (Bruker Biospin GmbH, Rheinstetten, Germany). Each peak was identified through Chenomx NMR suite 7.1 (Chenomx, Inc., Edmonton, AB, Canada) and Human Metabolome Database (www.hmdb.ca), and spectra were analyzed using Topspin 4.0.8 (Bruker Biospin GmbH).

### *2.2.7. Statistical analysis*

All experiments were performed in triplicate independently, except for electronic tongue and NMR analysis, which were 1 and 5 replicates, respectively. All Data were assessed using SAS software (version 9.4, SAS Institute Inc., Cary, NC) with statistical significance set at $P < 0.05$. Results were expressed as mean ± standard deviation. Statistical analysis was conducted using one-way analysis of variance (ANOVA) and Tukey's multiple comparison test was used to determine the difference between means. For NMR analysis and PCA, were performed using MetaboAnalyst 5.0 (www.metaboanalyst.ca). The data were Log-transformed and auto-scaled prior to multivariate and pathway analyses.

## 2.3. Results and discussion

### *2.3.1. Bactericidal effect of PAOA*

Figure. 2 presents the bactericidal effect of each PAOA against the three different pathogens. PAA and PAL showed ND values at all concentrations for the three pathogens. PAC showed a higher bactericidal effect than PAW, but a lower bactericidal effect than PAA and PAL against *S*. Typhimurium and *E. coli* O157:H7 ($P < 0.05$). Furthermore, the bactericidal effect of AA is significantly enhanced when combined with plasma compared to untreated AA ($P < 0.05$). When compared to DDW treatment, 0.5% and 1.0% AA treatments showed bactericidal effects of 1.05 and 2.67 log CFU/mL against *S*. Typhimurium, 0.63 and 1.07 log CFU/mL against *E. coli* O157:H7, and 1.81 and 3.14 log CFU/mL against *L. monocytogenes*, respectively. Conversely, 0.5% and 1.0% PAA showed ND values for all three pathogens. The synergistic effect between AA and plasma has been previously confirmed in other studies. In the study of Kang et al. (2022a), combined treatment of plasma and AA against *S*. Typhimurium exhibited approximately 5.71 log CFU/mL higher reduction effect than untreated AA. Among PAOA, only PAA demonstrated the highest bactericidal effect, along with a synergistic effect with plasma.

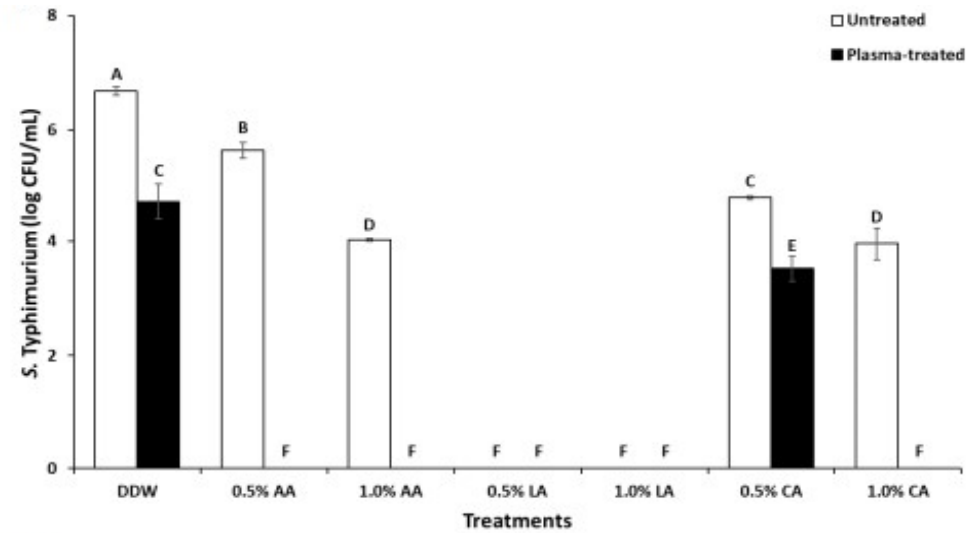
Figure. 3 shows the bactericidal effect of PAOA on pork loin inoculated with each pathogen. In the case of *S*. Typhimurium, the bactericidal effect of PAW was not significantly different when compared with the DDW ($P > 0.05$) (Figure. 3A). However, 0.5% and 1.0% PAA showed reduction levels of 2.44 and 2.55 log CFU/g,

respectively, compared to DDW, and significantly higher bactericidal effects than PAW ($P < 0.05$). In addition, 0.5% PAA demonstrated a reduction level of 1.47 log CFU/g higher than 0.5% AA and a significantly higher bactericidal effect ($P < 0.05$). PAL and PAC did not exhibit any differences when compared with the PAW ($P > 0.05$). For *E. coli* O157:H7, PAOA showed significantly higher bactericidal effects than DDW ($P < 0.05$) (Figure. 3B). Compared to the DDW treatment, 0.5% and 1.0% PAA exhibited reduction levels of 1.10 and 1.78 log CFU/g, respectively. Additionally, 0.5% and 1.0% PAL demonstrated reduction levels of 0.93 and 1.47 log CFU/g, respectively, and 0.5% and 1.0% PAC exhibited reduction levels of 0.85 and 1.10 log CFU/g, respectively. However, only 0.5% AA showed a significant difference in bactericidal effect based on the treatment or non-treatment of plasma ($P < 0.05$). Regarding *L. monocytogenes*, PAOA showed significantly higher bactericidal effects than PAW and DDW, but there was no significant difference in antibacterial activity based on the treatment or non-treatment of plasma ($P > 0.05$) (Figure. 3C). The bactericidal effect of PAOA against *L. monocytogenes* is likely due to the antibacterial activity of organic acid.

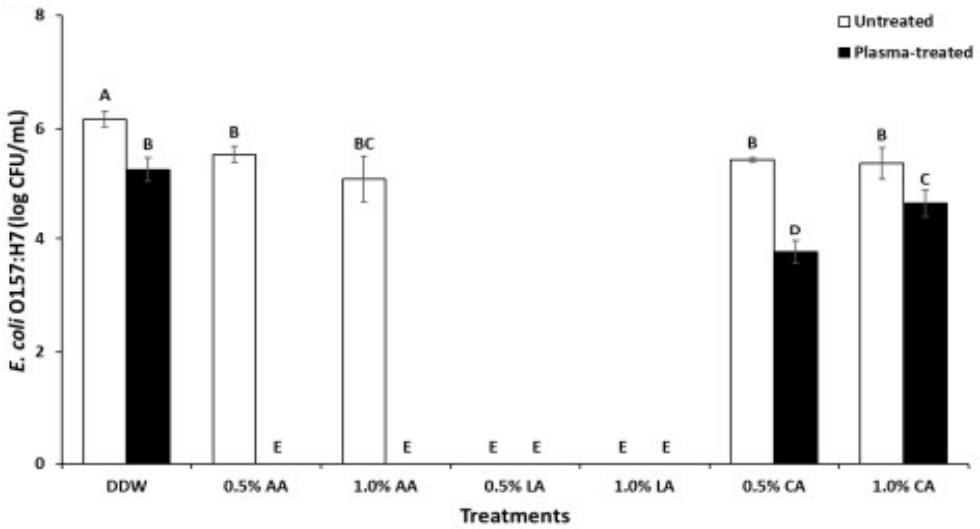
The PAW treatment had a significantly higher bactericidal effect than DDW treatment for the bacterial solution but showed no significant difference in bactericidal effect for pork loin inoculated with each pathogen. This may be due to the preferential reaction of the reactive species generated through plasma discharge with the pork loin, leading to a relatively reduced number of reactive species that can react with the pathogen present in the pork loin. Baek et al. (2020) reported that plasma treatment with organic matter reduces the number of reactive species that can

inactivate bacterial cells as the reactive species react with organic matter. These findings are consistent with other studies that treated PAW for organic matter (Xiang et al., 2019). In addition, the bactericidal effect of 0.5% PAA was significantly higher than 0.5% AA in *S*. Typhimurium and *E. col*i O157:H7 ($P < 0.05$), but no significant difference was observed in *L. monocytogenes* ($P > 0.05$). This is due to the structural differences between Gram-negative bacteria (*S*. Typhimurium and *E. coli* O157:H7) and Gram-positive bacteria (*L. monocytogenes*) (Yoo et al., 2021). Specifically, *L. monocytogenes* has a thick outer layer composed of peptidoglycan and requires a higher concentration of ROS for cell membrane disruption compared to the other two pathogens (Xu et al., 2018). Therefore, the bactericidal effect of plasma on *L. monocytogenes* was lower than that of other pathogens, and no significant difference between 0.5% AA and PAA was observed.

**(A)**


Untreated
Plasma-treated
S. Typhimurium (log CFU/mL)
8
6
4
2
0
A
C
B
F
D
F
F
F
F
F
C
E
D
F
DDW
0.5% AA
1.0% AA
0.5% LA
1.0% LA
0.5% CA
1.0% CA
Treatments

**(B)**


Untreated
Plasma-treated
E. coli O157:H7 (log CFU/mL)
8
6
4
2
0
A
B
B
E
BC
E
E
E
E
E
B
D
B
C
DDW
0.5% AA
1.0% AA
0.5% LA
1.0% LA
0.5% CA
1.0% CA
Treatments

**(C)**

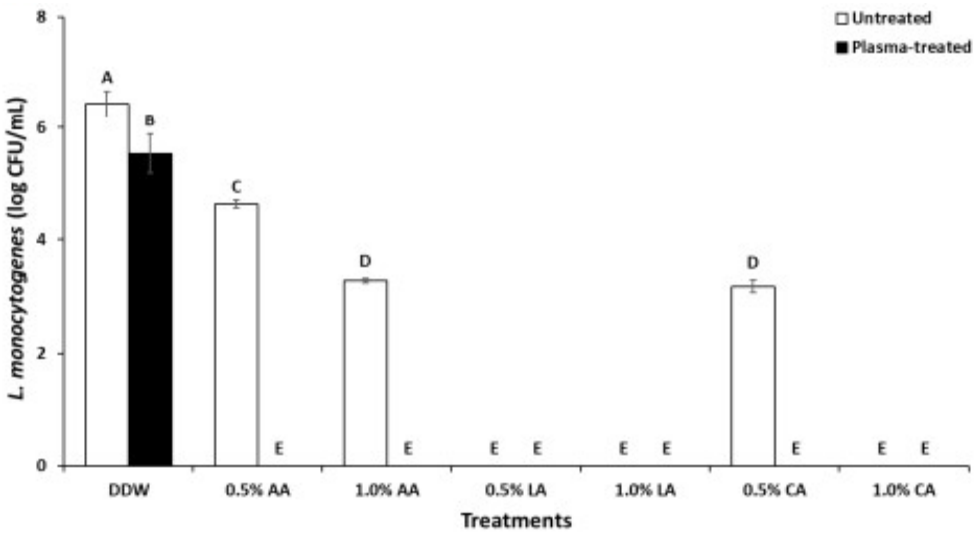
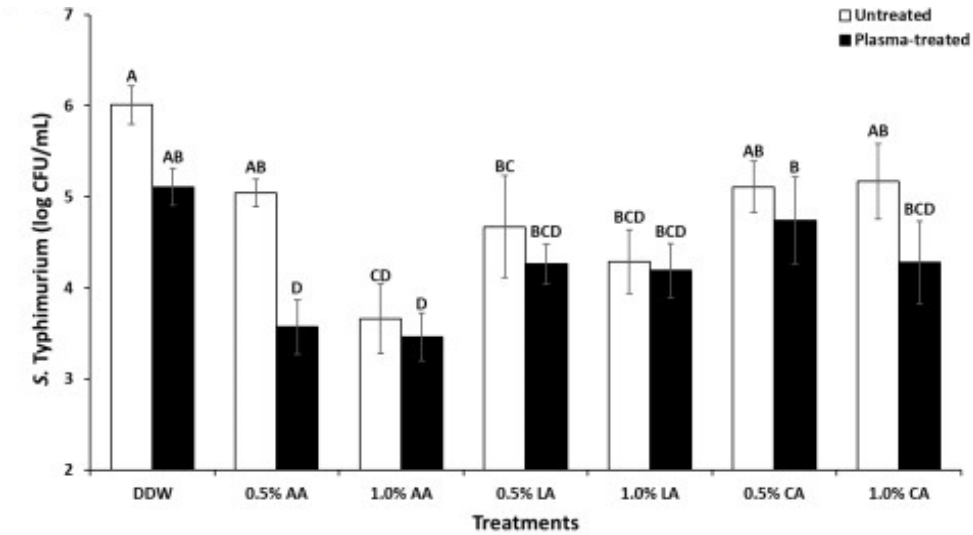
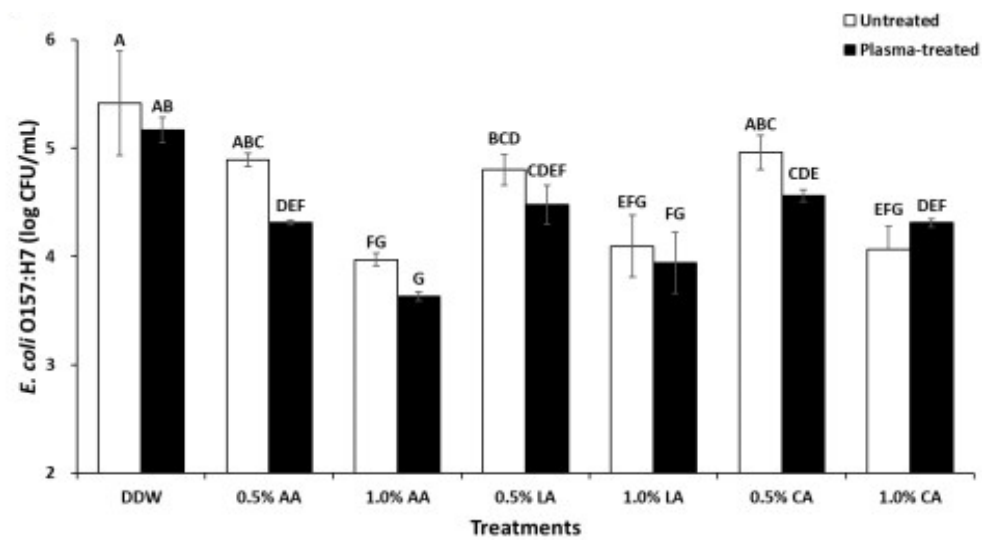



Figure 2. Bactericidal effect of DDW, organic acid, and plasma-activated organic acid on bacterial solution. *S.* Typhimurium (A), *E. coli* O157:H7 (B), and *L. monocytogenes* (C) survival in different treatments. DDW, deionized water; PAW, plasma-activated water; AA, acetic acid; LA, lactic acid; CA, citric acid; PAA, plasma-activated acetic acid; PAL, plasma-activated lactic acid; PAC, plasma-activated citric acid. Error bars represent standard deviation. [A-E]Different letters indicate a significant difference ($P < 0.05$) among the treatment.

**(A)**


Untreated
Plasma-treated
S. Typhimurium (log CFU/mL)
7
6
5
4
3
2
A
AB
AB
D
CD
D
BC
BCD
BCD
BCD
AB
B
AB
BCD
DDW
0.5% AA
1.0% AA
0.5% LA
1.0% LA
0.5% CA
1.0% CA
Treatments

**(B)**


Untreated
Plasma-treated
E. coli O157:H7 (log CFU/mL)
6
5
4
3
2
A
AB
ABC
DEF
FG
G
BCD
CDEF
EFG
FG
ABC
CDE
EFG
DEF
DDW
0.5% AA
1.0% AA
0.5% LA
1.0% LA
0.5% CA
1.0% CA
Treatments

**(C)**

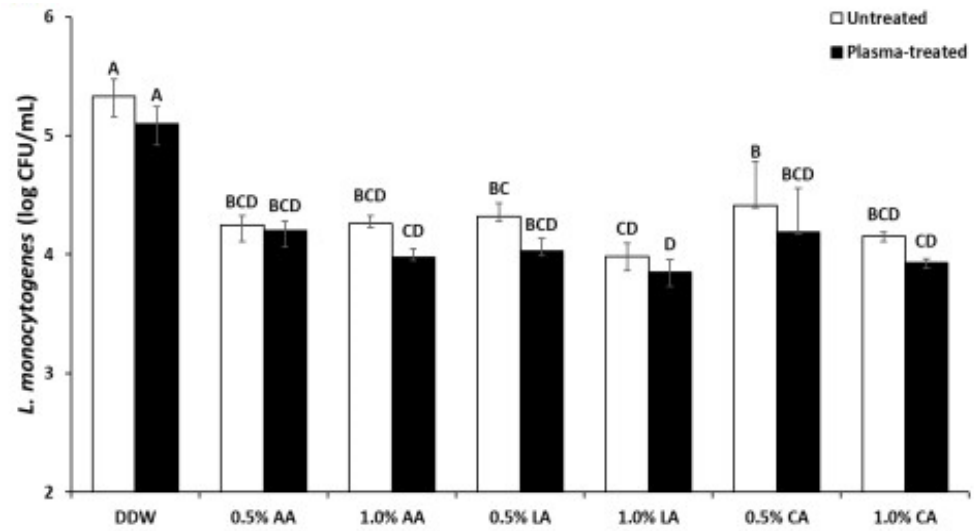



Figure 3. Bactericidal effect of DDW, organic acid, and plasma-activated organic acid on inoculated pork loin. *S.* Typhimurium (A), *E. coli* O157:H7 (B), and *L. monocytogenes* (C) survival in different treatments. DDW, deionized water; PAW, plasma-activated water; AA, acetic acid; LA, lactic acid; CA, citric acid; PAA, plasma-activated acetic acid; PAL, plasma-activated lactic acid; PAC, plasma-activated citric acid. Error bars represent standard deviation. [A-G]Different letters indicate a significant difference ($P < 0.05$) among the treatment.

### *2.3.2. Physicochemical properties of PAOA*

#### *2.3.2.1. pH and ORP*

PAOA treatments exhibited significantly lower pH values than PAW ($P < 0.05$) (Figure. 4A), which may be the reason for the higher bactericidal effect of PAOA compared to PAW. In pathogens, pH is a crucial environmental factor, and a low pH level can suppress microbial growth and cause bacterial cell death (Jin et al., 2018). In other words, the low pH of PAOA likely induced more stress on the cytoplasmic materials of each pathogen than PAW, leading to a higher bactericidal effect (Lund et al., 2020).

Furthermore, the PAOA treatments demonstrated significantly higher ORP values than PAW ($P < 0.05$) (Figure. 4B). These findings also support the higher bactericidal effect of PAOA compared to PAW. Higher ORP levels can increase microbial inhibition by inducing more oxidative stress on the bacterial cell membrane (Shen et al., 2016). In addition, the ORP of PAL did not exhibit a significant difference compared to LA ($P > 0.05$). This may be the reason why there was no synergistic effect observed in the combined treatment of LA and PAL.

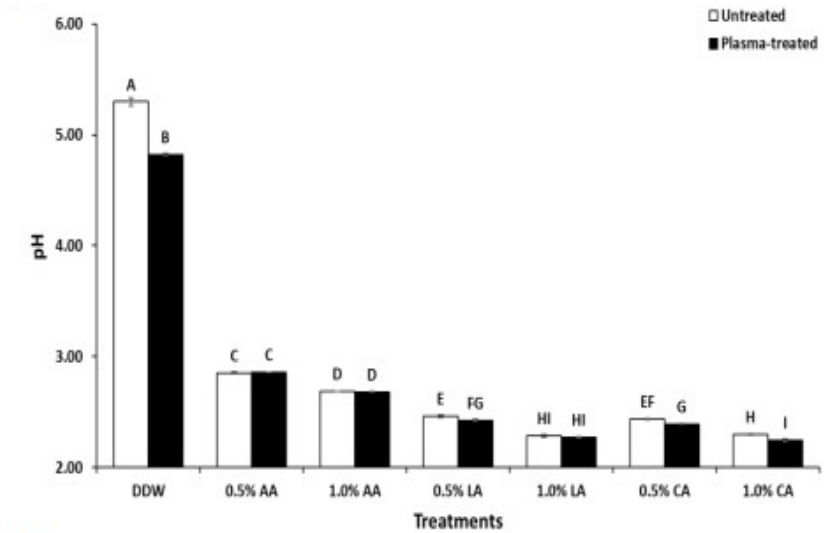
#### *2.3.2.2. ROS concentration*

$H_2O_2$ is a long-lived ROS that is produced during plasma discharge, and $H_2O_2$ dissolved in the treatment solution can inactivate pathogenic bacteria (Ma et al., 2020). All organic acids showed a significant increase in $H_2O_2$ concentration after

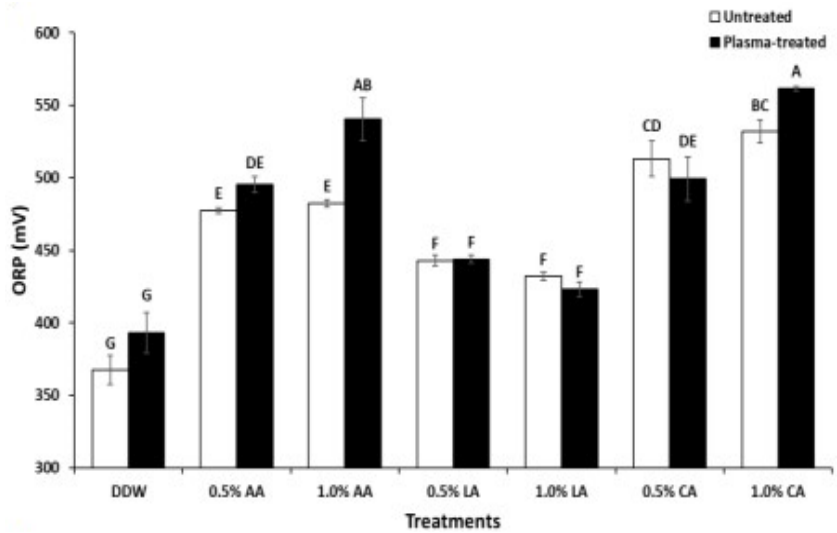
plasma treatment ($P < 0.05$) (Figure. 4C). Especially, PAOA except for 0.5% PAC, exhibited significantly higher $H_2O_2$ concentration than PAW ($P < 0.05$), which may have contributed to the higher bactericidal activity of PAOA than PAW. In particular, when 0.5% AA was combined with plasma treatment, the increase rate of $H_2O_2$ concentration (76.2% increase) was the highest among all organic acid and plasma combined treatment groups. This finding indicates that the combined treatment of 0.5% AA and plasma demonstrated a higher bactericidal effect compared to treatments using either method alone. It suggests that there may be a synergistic effect between the two treatments, which likely contributed to the observed increase in bactericidal activity.

Moreover, PAA treatment showed a significantly higher level of ozone concentration than other plasma-activated treatments ($P < 0.05$) (Figure. 4D). In particular, 0.5% AA significantly increased the ozone concentration by about 97% after plasma treatment, which is the highest level of increase among all treatments. Meanwhile, PAL and PAC exhibited significantly lower ozone concentrations than PAW. Thus, 0.5% AA showed the highest increase in ROS concentration among all treatments after plasma treatment, which may have led to the occurrence of a synergistic effect in combined treatment of plasma and 0.5% AA, resulting in significantly increased bactericidal effect.

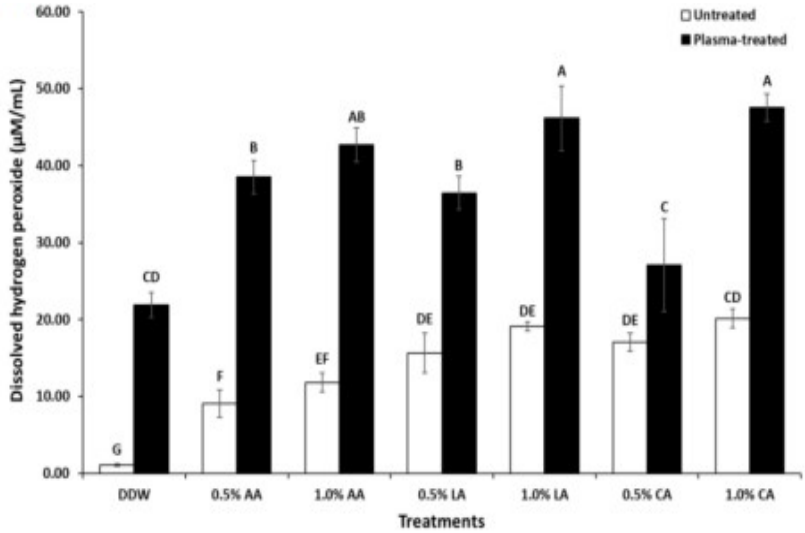
**(A)**


Untreated
Plasma-treated
6.00
5.00
4.00
3.00
2.00
pH
A
B
C
C
D
D
E
FG
HI
HI
EF
G
H
I
DDW
0.5% AA
1.0% AA
0.5% LA
1.0% LA
0.5% CA
1.0% CA
Treatments

**(B)**


Untreated
Plasma-treated
600
550
500
450
400
350
300
ORP (mV)
G
G
E
DE
E
AB
F
F
F
F
CD
DE
BC
A
DDW
0.5% AA
1.0% AA
0.5% LA
1.0% LA
0.5% CA
1.0% CA
Treatments

**(C)**




**(D)**

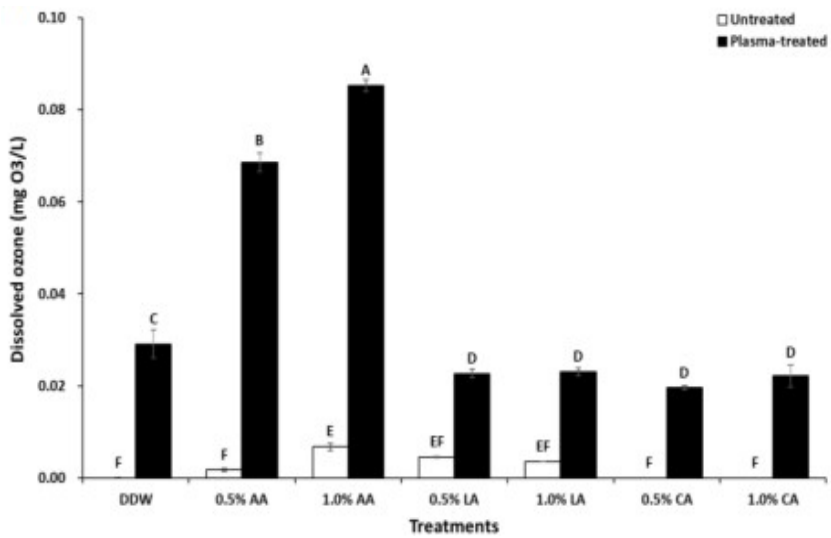



Figure 4. pH (A), Oxidation-reduction potential (ORP) (B), hydrogen peroxide concentration (C), and ozone concentration (D) of DDW, PAW, organic acid, and plasma-activated organic acid. DDW, deionized water treatment; PAW, plasma-activated water; AA, acetic acid; LA. lactic acid; CA, citric acid; PAA, plasma-activated acetic acid; PAL, plasma-activated lactic acid; PAC, plasma-activated citric

acid. Error bars represent standard deviation. [A-I]Different letters indicate a significant difference ($P < 0.05$) among the treatment.

### *2.3.3. Physicochemical properties of pork loin*

#### *2.3.3.1. pH*

The pH value of meat is a crucial factor that affects its freshness and sensory quality. The pH value of the PAW did not differ significantly from that of DDW ($P > 0.05$) (Table 1). In contrast, the organic acids and PAOA treatment showed significantly lower pH values than DDW and PAW ($P < 0.05$). The reduction in pH of pork loin treated with PAOA could be related to the use of each organic acid (Kang et al., 2022b). In addition, PAOA, with its relatively lower pH than the PAW, can inhibit pathogen growth, which could have contributed to the higher bactericidal effect of the PAOA than PAW treatment (Raftari et al., 2009). Some studies indicate that treating meat with a plasma-activated solution can lower its pH value due to reactive species dissolved in the solution (Qian et al., 2021b). However, in this study, there was no significant difference between the organic acid and the PAOA treatment in terms of pH ($P > 0.05$).

#### *2.3.3.2. Surface color*

In Table 1, no significant difference in $L^*$- and $a^*$-value was observed when comparing PAW and PAA with DDW ($P > 0.05$). This finding is consistent with Kang et al. (2022b) that PAA treatment on drumstick showed no significant difference in $L^*$- and $a^*$-value when compared to the DDW treatment. Although no significant difference was observed between the $b^*$-value of PAA and DDW in their study (Kang

et al., 2022b), $b^*$-value was significantly decreased after PAA treatment ($P < 0.05$) in this study. However, this decrease is considered negligible for pork loin, as there was no significant difference in the photographs of PAA and DDW (Figure. 5). Meanwhile, PAL showed significant differences in $L^*$- and $a^*$-value when compared to DDW, and PAC showed significant differences in $L^*$-, $a^*$-, and $b^*$-value ($P < 0.05$). This indicates that the PAL and PAC treatment induced more changes than other plasma-activated treatments, as evidenced by the significantly different photographs of PAL and PAC compared to DDW.

Table 1. pH value and surface color of pork loin treated with organic acid, PAW, and plasma-activated organic acid

| Treatments | pH | $L^*$ | $a^*$ | $b^*$ |
|---|---|---|---|---|
| DDW | $5.82 \pm 0.02^{a}$ | $54.72 \pm 1.38^{c}$ | $4.53 \pm 0.23^{bc}$ | $11.64 \pm 0.11^{a}$ |
| PAW | $5.76 \pm 0.03^{a}$ | $55.07 \pm 0.24^{c}$ | $4.82 \pm 0.06^{b}$ | $10.05 \pm 0.06^{d}$ |
| AA | $5.18 \pm 0.04^{b}$ | $58.01 \pm 0.18^{ab}$ | $4.01 \pm 0.24^{cd}$ | $9.97 \pm 0.49^{d}$ |
| LA | $5.22 \pm 0.04^{b}$ | $50.36 \pm 1.96^{d}$ | $4.65 \pm 0.35^{b}$ | $11.19 \pm 0.07^{ab}$ |
| CA | $5.32 \pm 0.07^{b}$ | $59.73 \pm 0.29^{a}$ | $3.63 \pm 0.25^{d}$ | $10.41 \pm 0.09^{cd}$ |
| PAA | $5.24 \pm 0.10^{b}$ | $57.24 \pm 0.63^{abc}$ | $4.79 \pm 0.11^{b}$ | $10.39 \pm 0.39^{cd}$ |
| PAL | $5.20 \pm 0.05^{b}$ | $51.81 \pm 0.323^{d}$ | $5.80 \pm 0.08^{a}$ | $11.04 \pm 0.29^{abc}$ |
| PAC | $5.18 \pm 0.07^{b}$ | $56.32 \pm 0.52^{bc}$ | $3.88 \pm 0.03^{d}$ | $10.48 \pm 0.22^{bcd}$ |

DDW, deionized water treatment; PAW, plasma-activated water; AA, 0.5% acetic acid; LA. 0.5% lactic acid; CA, 0.5% citric acid; PAA, 0.5% plasma-activated acetic acid; PAL, 0.5% plasma-activated lactic acid; PAC, 0.5% plasma-activated citric acid.

[1]All values represent the mean ± standard deviation.

[2]$L^*$: Lightness; $a^*$: redness; $b^*$: yellowness.

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

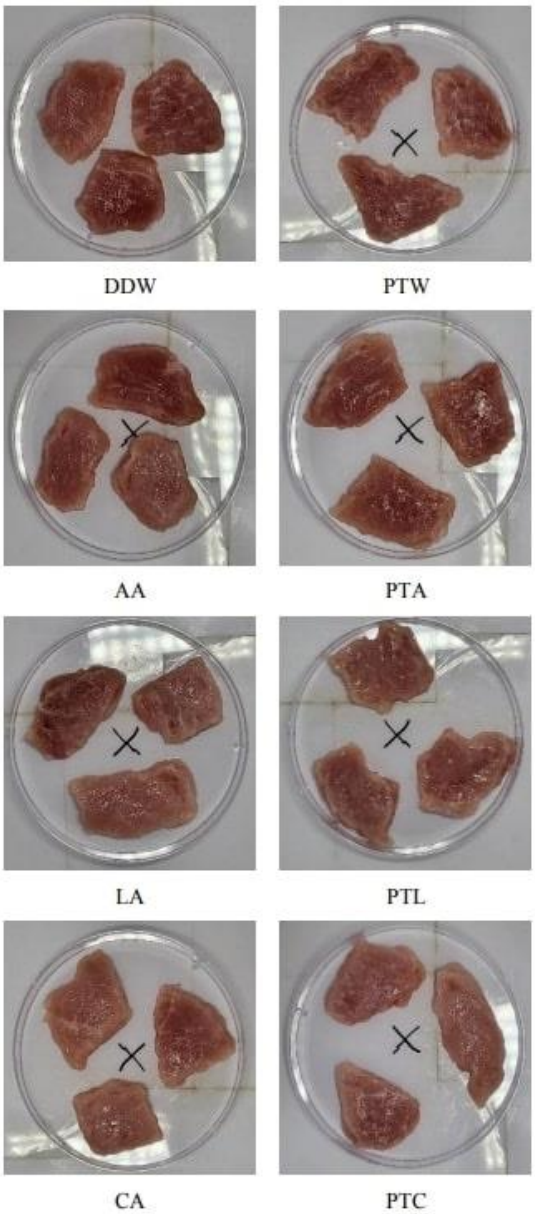



Figure 5. Photograph of surface of pork loin treated by various treatment solution. DDW, deionized water treatment; PAW, plasma-activated water; AA, 0.5% acetic acid; LA. 0.5% lactic acid; CA, 0.5% citric acid; PAA, 0.5% plasma-activated acetic acid; PAL, 0.5% plasma-activated lactic acid; PAC, 0.5% plasma-activated citric acid.

### *2.3.4. Storage stability*

#### *2.3.4.1. Lipid oxidation*

The malondialdehyde concentration was evaluated as the TBARS value to determine the effect of plasma treatment on the lipid oxidation of pork loin (Table 2). The TBARS value of PAW showed no significant difference compared to DDW during the storage period ($P > 0.05$). PAL showed no significant difference from DDW on days 0 and 6 but showed significantly higher TBARS values on day 3 ($P < 0.05$). However, PAA and PAC showed no significant difference or lower TBARS values than DDW during the storage period. In particular, the TBARS value of PAA and PAC was significantly lower than that of PAOA on day 6. This result may be due to the influence of reactive species generated through plasma and organic acid on pork loin. During the storage period, AA, LA, and CA can inhibit the production of oxidative by-products that can form MDA by inhibiting microbial growth in meat (Kang et al., 2002). This can be confirmed through the organic acid treatment group (except for 6 days AA), which showed no significant difference or lower TBARS value than DDW. On the other hand, some studies suggest that plasma treatment of meat increases lipid oxidation. Jayasena et al. (2015) reported that pork and beef increased TBARS values with increasing plasma treatment time. However, in this study, the results were contrary to the above experiment. This is because nitrite generated through plasma discharge acts as an antioxidant, such as binding to irons in the myoglobin of pork loin or removing radicals that cause lipid peroxidation (Yong et al., 2019). The DBD plasma system used in this study produced nitrite

during plasma discharge (Figure. 6), and then nitrite was dissolved in the plasma-activated solution to act as an antioxidant. In addition, several studies have reported that inhibition of lipid oxidation is similar to the results of this study when plasma and organic acids are combined. Kang et al. (2022b) showed inhibition in TBARS value when AA and plasma were combined with chicken drumstick and breast, and Qian et al. (2021b) and Qian et al. (2019) also reported lower lipid oxidation than DDW when combined treatment of LA and plasma were applied to chicken drumstick and beef, respectively. Therefore, combined treatment of organic acid and plasma did not have a negative effect on the lipid oxidation of pork loin.

Table 2. Lipid oxidation of pork loin treated with PAW, organic acid and plasma-activated organic acid

| Treatment | TBARS value (mg malondialdehyde /kg) | | |
|---|---|---|---|
| | Storage period (days) | | |
| | 0 | 3 | 6 |
| DDW | $0.078 \pm 0.004^{ay}$ | $0.080 \pm 0.004^{by}$ | $0.106 \pm 0.010^{bcx}$ |
| PAW | $0.078 \pm 0.001^{ay}$ | $0.080 \pm 0.001^{by}$ | $0.091 \pm 0.004^{cdx}$ |
| AA | $0.078 \pm 0.005^{aby}$ | $0.079 \pm 0.005^{by}$ | $0.126 \pm 0.004^{ax}$ |
| LA | $0.067 \pm 0.005^{by}$ | $0.079 \pm 0.004^{by}$ | $0.100 \pm 0.008^{bcdx}$ |
| CA | $0.078 \pm 0.002^{ay}$ | $0.083 \pm 0.003^{by}$ | $0.116 \pm 0.013^{abx}$ |
| PAA | $0.071 \pm 0.001^{aby}$ | $0.075 \pm 0.004^{bxy}$ | $0.080 \pm 0.004^{dx}$ |
| PAL | $0.071 \pm 0.001^{abz}$ | $0.100 \pm 0.001^{ay}$ | $0.112 \pm 0.007^{abx}$ |
| PAC | $0.071 \pm 0.002^{aby}$ | $0.084 \pm 0.004^{bx}$ | $0.085 \pm 0.003^{dx}$ |

DDW, deionized water treatment; PAW, plasma-activated water; AA, 0.5% acetic acid; LA. 0.5% lactic acid; CA, 0.5% citric acid; PAA, 0.5% plasma-activated acetic acid; PAL, 0.5% plasma-activated lactic acid; PAC, 0.5% plasma-activated citric acid.

[1]All values represent the mean ± standard deviation.

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

[x-z]Different letters within the same row differ significantly ($P < 0.05$).

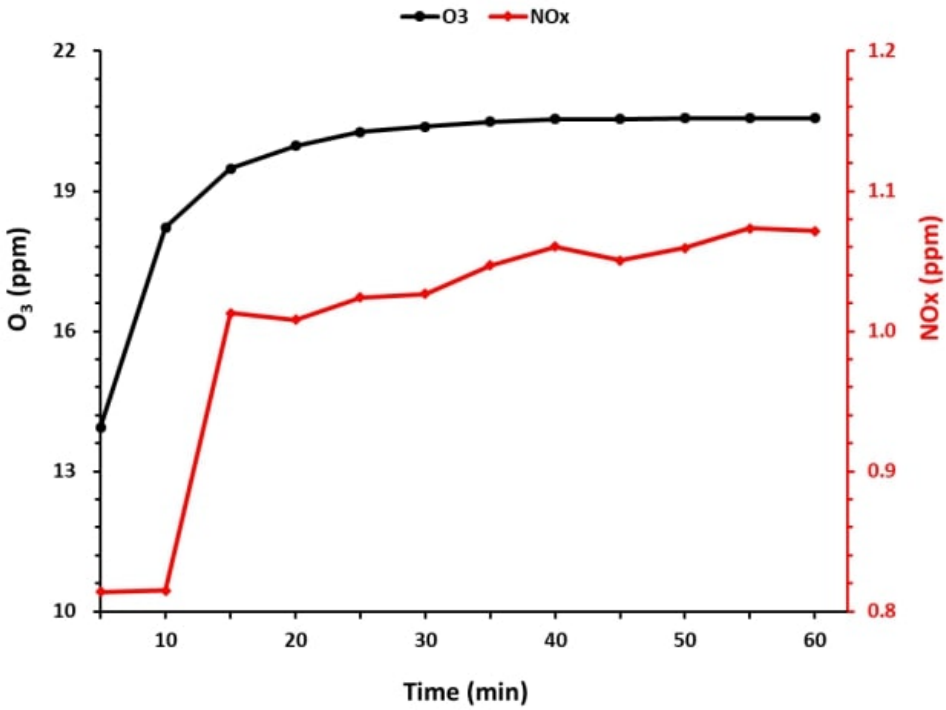



Figure 6. Concentrations of ozone and NOx generated by time during Bead-type DBD plasma discharge.

### *2.3.4.2. Protein oxidation*

Carbonyl content was measured to confirm the protein oxidation in pork loin treated with plasma-activated treatments (Table 3). The PAW treatment resulted in higher carbonyl content during the storage period than DDW. This is because reactive species generated through plasma discharge oxidize the side chains of amino acid residues (Luo et al., 2022). However, when organic acid and plasma were combined in this study, different result were observed. PAA showed a lower carbonyl content than DDW and PAW on all storage days. Compared to DDW, PAL showed no significant difference compared to DDW on day 0 and 3 but had lower carbonyl content on day 6. PAC had higher carbonyl contents than DDW on day 0, but there was no significant difference on days 3 and 6. These results could be attributed to the effect of organic acids on pork loin. Organic acids at appropriate concentrations can chelate pro-oxidant metals that cause protein oxidation, and reduce the exposure of amino acid residues by inhibiting the swelling of protein molecules (Lin et al., 2022). Therefore, pork loin treated with PAOA can inhibit the level of protein oxidation that occurs during PAW treatment.

Table 3. Protein oxidation of pork loin treated with organic acid, PAW, and plasma-activated organic acid

| Treatment | Carbonyl content (nmol/mg) | | |
|---|---|---|---|
| | Storage period (days) | | |
| | 0 | 3 | 6 |
| DDW | 2.15 ± 0.02$^{cdz}$ | 2.53 ± 0.01$^{aby}$ | 2.91 ± 0.04$^{bx}$ |
| PAW | 2.52 ± 0.09$^{ay}$ | 2.65 ± 0.03$^{ay}$ | 3.01 ± 0.03$^{ax}$ |
| AA | 2.23 ± 0.00$^{bcz}$ | 2.54 ± 0.05$^{aby}$ | 2.84 ± 0.02$^{cdx}$ |
| LA | 2.25 ± 0.01$^{bcz}$ | 2.29 ± 0.01$^{dy}$ | 2.81 ± 0.02$^{cdx}$ |
| CA | 2.13 ± 0.01$^{cdz}$ | 2.35 ± 0.04$^{cdy}$ | 2.80 ± 0.02$^{dx}$ |
| PAA | 1.97 ± 0.09$^{dz}$ | 2.14 ± 0.01$^{ey}$ | 2.56 ± 0.01$^{ex}$ |
| PAL | 2.15 ± 0.01$^{cdz}$ | 2.52 ± 0.02$^{aby}$ | 2.53 ± 0.00$^{ex}$ |
| PAC | 2.40 ± 0.19$^{aby}$ | 2.47 ± 0.13$^{bcy}$ | 2.86 ± 0.02$^{bcx}$ |

DDW, deionized water treatment; PAW, plasma-activated water; AA, 0.5% acetic acid; LA. 0.5% lactic acid; CA, 0.5% citric acid; PAA, 0.5% plasma-activated acetic acid; PAL, 0.5% plasma-activated lactic acid; PAC, 0.5% plasma-activated citric acid.

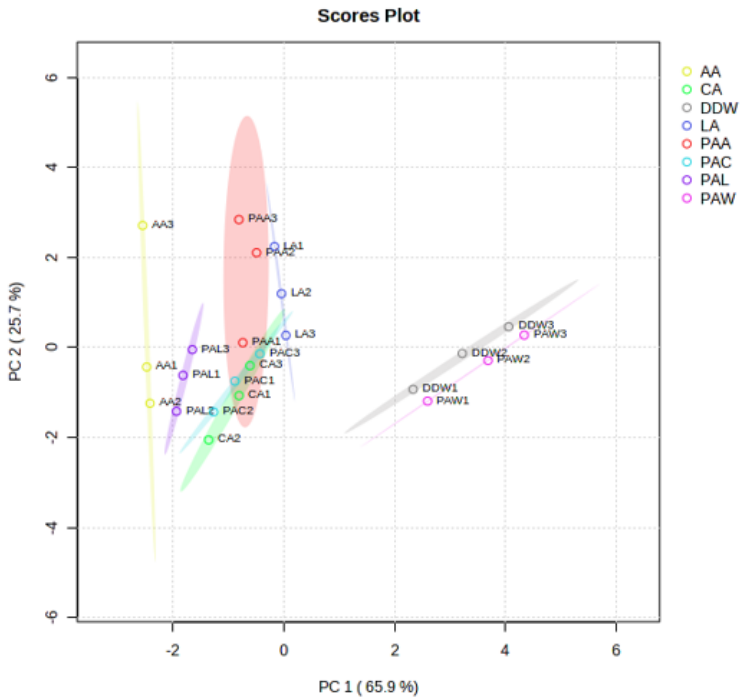
[1]All values represent the mean ± standard deviation.

[a-e]Different letters within the same column differ significantly ($P < 0.05$).

### *2.3.5. Taste attributes by electronic tongue*

To analyze the effect of each treatment solution on the taste attributes of the pork loin an electronic tongue was employed. The taste attributes of each treatment group were profiled using PCA (Figure. 7A). Among all treatments, the PAW exhibited similar taste attributes to DDW, while the organic acid and PAOA showed a significant difference, which is evident from the difference in each taste attribute score shown in Figure. 7B. Moreover, PAA showed a smaller difference from DDW in terms of sourness (AHS) and saltiness (CTS) than PAL and PAC and exhibited the highest value for umami value among all treatments. The high umami intensity of PAA treatment could be attributed to IMP. Table 4 indicates that the PAA treatment has a higher IMP content than other treatments. Thus, the PAA treatment had taste attributes relatively similar to those of the DDW treatment compared to other PAOA and exhibited the most excellent result among all treatments in terms of umami taste.

**(A)**


Scores Plot
AA
CA
DDW
LA
PAA
PAC
PAL
PAW
PC 2 ( 25.7 %)
PC 1 ( 65.9 %)
AA3
PAA3
LA1
PAA2
LA2
LA3
PAA1
PAC3
CA3
PAL3
AA1
PAL1
PAC1
CA1
AA2
PAL2
PAC2
CA2
DDW3
PAW3
DDW2
PAW2
DDW1
PAW1

**(B)**

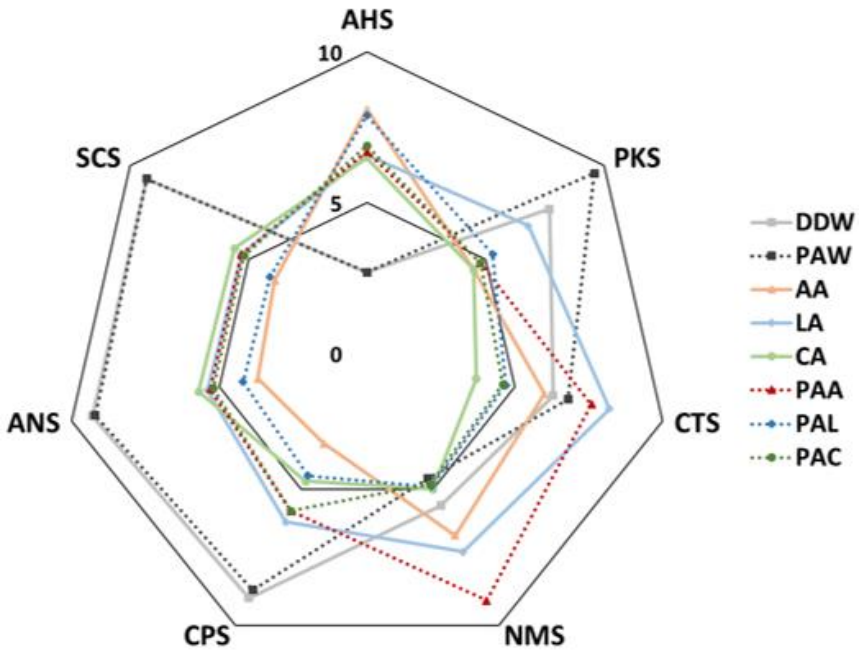

AHS
10
5
0
PKS
CTS
NMS
CPS
ANS
SCS
DDW
PAW
AA
LA
CA
PAA
PAL
PAC

Figure 7. Principal component analysis plot (A), Spider plot for taste attributes (B) of organic acid, PAW, and plasma-activated organic acid. DDW, deionized water; PAW, plasma-activated water; AA, acetic acid; LA, lactic acid; CA, citric acid; PAA, plasma-activated acetic acid; PAL, plasma-activated lactic acid; PAC, plasma-activated citric acid; AHS, sour taste; CTS, salty taste; NMS, umami taste; PTS, sweet taste; ANS, bitter taste.

Table 4. List of quantified Inosine 5′-monophosphate of organic acid, PAW, and plasma-activated organic acid treated pork loin by [1]H NMR analysis (mg/100g)

| Treatment | Item |
|---|---|
| | IMP |
| DDW | 968.61 ± 32.17[ab] |
| PAW | 905.71 ± 32.10[b] |
| AA | 975.67 ± 66.45[ab] |
| LA | 964.01 ± 38.04[ab] |
| CA | 955.42 ± 48.64[ab] |
| PAA | 1025.12 ± 24.32[a] |
| PAL | 944.00 ± 28.94[b] |
| PAC | 938.25 ± 14.81[b] |

DDW, deionized water treatment; PAW, plasma-activated water; AA, 0.5% acetic acid; LA. 0.5% lactic acid; CA, 0.5% citric acid; PAA, 0.5% plasma-activated acetic acid; PAL, 0.5% plasma-activated lactic acid; PAC, 0.5% plasma-activated citric acid.

[1]All values represent the mean ± standard deviation.

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

## 2.4. Conclusion

The bactericidal effect of PAOA against pork loin inoculated with pathogens was found to be higher than that of PAW. In particular, 0.5% PAA showed higher antibacterial activity against *S*. Typhimurium and *E. coli* O157:H7 compared to 0.5% AA, unlike other PAOA. It suggested a synergistic effect between plasma and organic acid treatment. PAA showed a similar meat color to DDW, minimal lipid oxidation, and reduced protein oxidation during storage. Additionally, PAA exhibited the highest umami taste level surpassing all other treatments. Hence, PAA can be considered an advanced technology in PAW, useful for enhancing microbial safety and oxidative stability in the meat industry.

# Chapter III.

# Effect of plasma-activated organic acids on different chicken cuts inoculated with *Salmonella* Typhimurium and *Campylobacter jejuni* and their antioxidant activity

This manuscript will be published in elsewhere as partial fulfillment of the Master's program of Hag Ju Lee.

## 3.1. Introduction

Chicken is one of the most popular meats as it is rich sources of protein for human consumption (Agyemang et al., 2021; Baek et al., 2020). In addition, chicken meat has the advantage of being lower in fat content and price, and it is less restrictions by religious dietary practices compared to red meats (Ma et al., 2022). However, due to its nutrient-rich composition, chicken meat is susceptible to microbial contamination. Microorganisms can contaminate chicken meat during its production, distribution, and consumption, leading to rapid food spoilage and potential

foodborne illnesses (Kang et al., 2022b). *Salmonella* Typhimurium and *Campylobacter jejuni*, are the most representative pathogens in chicken, which can cause food poisoning, such as salmonellosis and campylobacteriosis (Hatanaka et al., 2020; Kang et al., 2022a; Lin, 2019). Therefore, it is essential to develop efficient method for controlling microorganisms to ensure the safe chicken meat consumption.

Various non-thermal technologies (e.g. ultrasonication, irradiation, and high-pressure processing) have been attempted to control microorganisms in chicken, without heat denaturation and/or further quality deterioration (González-González et al., 2021; Zhuang et al., 2019). Plasma is one of the non-thermal technologies, which include the ionized gas composed of different reactive species (e.g. ion, electron, free radical, and UV photons) (Lee et al., 2011). It can efficiently inactivate microorganisms; however, plasma has limitations in industrial application due to its low penetration depth and non-uniform treatment (Chen et al., 2019; Domonkos et al., 2021). For these reasons, several studies have been conducted to expend its application (Baek et al., 2020; Heo et al., 2021; Jayasena et al., 2015). Among them, PAW offers advantages as it is easy application to food in various forms, mass production feasibility, and cost-effectiveness (Gao et al., 2022; Zhou et al., 2020). PAW is defined as water containing effective reactive species for microbial inactivation (Astorga et al., 2022). It has been approved for its effect on the different types of meat including chicken (Gao et al., 2022). However, PAW has limitations when treating materials containing organic matter. The presence of organic matter can interfere with the reaction of reactive species in PAW as it can alter the physicochemical characteristics of PAW (Baek et al., 2020; Xiang et al., 2019). In

addition, when PAW is applied, it can increase lipid oxidation (Jayasena et al., 2015; Kim et al., 2013)

To address some of the limitations of PAW, this study aimed to develop PAOA by combining plasma treatment with organic acids. Organic acids are widely recognized disinfectants used for food decontamination (Cruz-Romero et al., 2013). In this study, we selected lactic acid and gallic acid due to their demonstrated antibacterial and antioxidant activities (Asnaashari et al., 2014; Kang et al., 2022; Kim, 1997; Mohamed & Abdel-Naeem, 2018; Tian et al., 2022). Previous studies have investigated the utilization of PAOA in chicken meat (Kang et al., 2022b; Qian et al., 2021). However, there is limited research on the application of PAOA using gallic acid and/or its combination with lactic acid. Furthermore, there is a lack of studies in the bactericidal effect of PAOA on *C. jejuni*. Therefore, the objective of this study was to investigate the combined effect of plasma and organic acids on bactericidal reduction in chickens and their oxidative stability during the storage period.

## 3.2. Materials and methods

### *3.2.1 Bacterial solution preparation*

*S.* Typhimurium (ATCC 14411) and *C. jejuni* (NCCP 11192 were cultured using Nutrient Broth (Difco, Detroit, Mich, USA) and Muller Hinton Broth (Sigma-Aldrich, St. Louis, MO, USA), respectively. Then, the broths were centrifuged at 4,001 ×g at 4°C for 10 min (Combi 514R, Hanil, Incheon, Korea). The supernatant was discarded, and the bacterial pellets were resuspended in 0.85% NaCl. This process was repeated twice. The final concentration of the bacterial solution was adjusted to $10^5$-$10^6$ CFU/mL by appropriate dilution with 0.85% NaCl.

### *3.2.2. Sample preparation*

#### *3.2.2.1. PAOA*

For the preparation of PAOA, the atmospheric pressure dielectric barrier discharge plasma was used in this study. The container is made of zirconium material, and 1 L beaker was placed inside with a distance of 12 cm from its electrode. The beaker was filled with 200 mL of 1% LA, GA, and LGA (1:1 v/v) in distilled water. Then, plasma was treated on the organic acids at 10 kHz and 4.0 kVpp for 60 min and plasma-activated LA, GA, and LGA were obtained for further applications (PAL, PAG, and PLGA, respectively). All organic acids (9.9 mL) prepared were inoculated with the bacterial solutions of *S.* Typhimurium and *C. jejuni* (0.1 mL), respectively. The mixture was allowed to react at room temperature for 10 min and used for the analyses.

#### *3.2.2.2. Chicken meat treated with PAOA*

Chickens were purchased from a local market (Seoul, Korea) and divided to breasts and drumsticks. To eliminate microorganism present in chicken meat, the samples were wiped with a food disinfectant (Jinro-Distillers, Ansan, Korea) and exposed to ultraviolet light for 30 min. Then, all the sterilized meat was uniformly cut into pieces of equal size (30 × 30 × 5 mm; 5.00 ± 0.05 g) using a sterilized knife, and 0.1 mL of bacterial solutions of *S*. Typhimurium and *C. jejun*i was inoculated onto each piece. The inoculated samples were dried at room temperature for microbial attachment and immersed in the prepared organic acids and PAOA for 10 min. The treatment time was set based on our preliminary study. After the treatment, the samples were stored at 4°C for 12 days and obtained 6 day-interval for further analyses.

### *3.2.3. Antibacterial effect*

Serial dilutions of PAOA and chicken meat were performed using 0.85% NaCl and their final dilutes with *S*. Typhimurium and *C. jejuni* (0.1 mL) were spread onto Xylose Lysine Deoxycholate agar plates (Difco, Detroit, Mich, USA) and Muller Hinton Agar (Sigma-Aldrich, St. Louis, MO, USA), respectively. Prior to the dilution, chicken meat (5 g) was transferred into a sterile bag containing 0.85% NaCl (45 mL). The remaining bacteria were detached from chicken meat using a stomacher for 2 min (Bag Mixer® 400P, Interscience Co, St. Nom la Bretèche, France). The agar plates were incubated at 37°C for 48 h and viable cells were

expressed as Log CFU/mL for PAOA and Log CFU/g for chicken meat.

### *3.2.4 Antioxidant activity*

#### *3.2.4.1. Total phenolic contents*

Total phenolic contents were measured by the Folin-Ciocalteu's method (Subramanian et al., 1965). In order to determine the total phenolic contents of PAOA, a mixture comprising 0.1 mL of the treatment solution and 0.2 mL of Folin-Ciocalteu reagent (Sigma-Aldrich, St. Louis, MO, USA) was prepared. Subsequently, 3 mL of 5% sodium carbonate (Duksan Pure Chem, Ansan, Korea) was added to the mixture. The resulting solution was thoroughly vortexed and incubated in the dark at 23°C for 2 h. Following incubation, the absorbance was measured at 765 nm using a spectrophotometer (SpectroMax M2e, Molecular Devices, Sunnyvale, CA). The obtained results were quantified based on a standard curve generated using gallic acid and expressed as mg gallic acid equivalent per mL (mg GAE/mL).

#### *3.2.4. $ABTS^+$ reducing activity*

Chicken breast and drumstick (3 g), respectively, were extracted by homogenizing with 15 mL of DDW for 1 min (T25 Basic, Ika Co., Staufen, Germany). The working solution of $ABTS^+$ was prepared by combining 14 mM of 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium with 4.9 mM of potassium persulfate in a 1:1 ratio (v/v). The working solution was diluted with

ethanol to achieve an absorbance value of 0.70 ± 0.02 at 734 nm using a spectrophotometer (SpectroMax M2e, Molecular Devices). Then, 3 mL of the working solution was mixed with 20 μL of the solution and chicken meat extract. The mixture was incubated at room temperature in the dark room for 10 min and centrifuged at 2,268 ×g, 4°C for 5 min (Continent 512R, Hanil Co., Ltd., Incheon, Korea). Then, their absorbance was measured (SpectroMax M2e, Molecular Devices) and calculated based on Trolox as standard and expressed as mmol Trolox equivalent per g (mM TE/g).

### *3.2.4.3. DPPH radical scavenging activity*

For DPPH analysis, DPPH was added to the samples. The extraction of chicken meat was conducted following the procedure described in section 2.4.2. The mixture was vigorously vortexed and allowed to react for 30 min in the dark at room temperature. Then, the samples were centrifuged at 2,265 ×g at 4°C for 15 min (Continent 512R, Hanil Co) and their absorbance was measured at 517 nm using a spectrophotometer (SpectroMax M2e, Molecular Devices). The obtained absorbance values were calculated based on Trolox as standard and expressed as mmol Trolox equivalent per g (mM TE/g).

### *3.2.4.4. TBARS*

TBARS values were measured to assess lipid oxidation in chicken meat during

12 days of storage. After adding 15 mL of DDW and 50 μL of butylated hydroxy toluene to 5 g of the sample treated with each treatment solution, the samples were homogenized for 30 sec (T25 Basic, Ika Co). The homogenized samples were centrifuged at 2,265 ×g at 4°C for 15 min (Continent 512R, Hanil Co), and the supernatant is filtered. Next, 2 mL of the homogenized sample was mixed with 4 mL of 20 mM 2-thiobarbituric acid and the mixture was heated at 90°C for 30 min using a water bath. After cooling the samples for 15 min, the mixture was vortexed and centrifuged at 2265 ×g for 15 min (Continent 512R, Hanil Co). Then, the absorbance was measured at 532 nm using a spectrophotometer (SpectroMax M2e, Molecular Devices). The TBARS value was expressed as mg MDA/kg of meat sample.

### *3.2.5. Statistical analysis*

All experiments were independently performed in triplicate. The data were analyzed using SAS software (version 9.4, SAS Institute Inc., Cary, NC, USA) with statistical significance set at $P < 0.05$. Results were expressed as mean values and standard error of the mean. Statistical analysis was conducted using two-way analyses of variance and Tukey's multiple comparison test.

## 3.3. Results and discussion

### *3.3.1. PAOA*

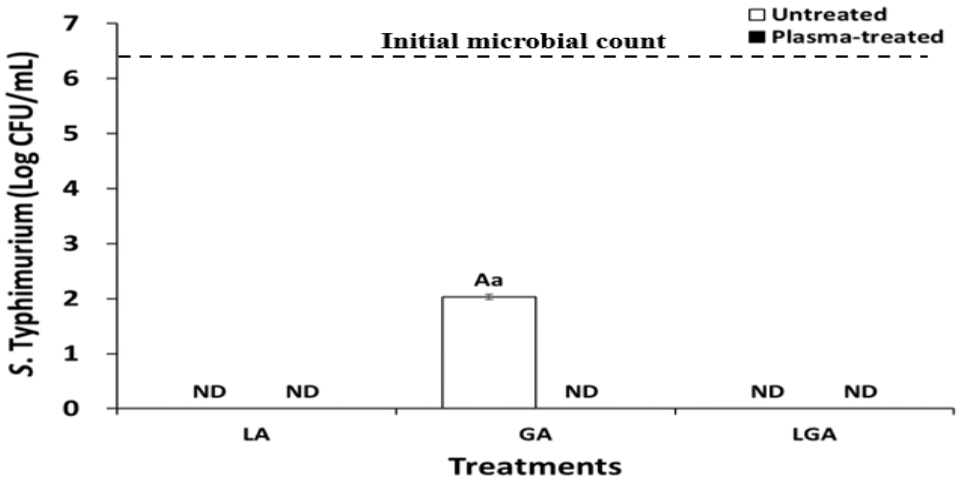
#### *3.3.1.1 Antibacterial effect*

In bacterial solution, the initial numbers of *S.* Typhimurium and *C. jejuni* were 6.37 and 5.54 Log CFU/mL, respectively. When organic acids and PAOA were treated on the bacterial solutions, the LA and LGA exhibited a higher bactericidal effect than GA against *S.* Typhimurium and *C. jejuni*, regardless of plasma treatment (Figure. 8). In details, LA and LGA sterilized *S.* Typhimurium with a reduction of 6.37 Log CFU/mL, while GA showed a microbial reduction of 4.34 Log CFU/mL. In the case of *C. jejuni*, their reduction was the highest in LGA, followed by LA and GA. For both pathogens, the use of LA could occur certain damage to the cell membrane and intracellular enzymes and proteins of microorganisms (Zhou et al., 2023), therefore, our result indicated the higher antibacterial effect by mainly LA addition. In other previous studies with organic acids, Jyung et al. (2023) and Stanojević-Nikolić et al. (2015) also reported the highest antibacterial effect of LA on different bacteria, including *Escherichia*, *Salmonella Enteritidis*, *Staphylococcus aureus*, *Listeria monocytogenes*, and *Bacillus cereus*.

When the organic acid and plasma were combined, the bactericidal effect of PAOA was increased against both pathogens, except for LA and LGA for *S.* Typhimurium (Figure. 8). We did not observe significant changes in LA and LGA for *S.* Typhimurium as LA itself could sterilize all inoculated bacteria first. However, several studies had demonstrated that combined treatment of LA and plasma can

enhance the antibacterial effect (Qian et al., 2019; Yadav et al., 2022). In this study, the presence of LA and LGA could have potentially improved the bactericidal effect when combined with plasma treatment, particularly if the initial numbers of *S*. Typhimurium were higher. It appears that the PAOA exhibited a synergistic interaction between the organic acids and plasma, likely due to the generation of ROS (Kang et al., 2022a; Qian et al., 2021). In previous study, PAOA can produce ROS composed of hydrogen peroxide, hydroxyl radical, and ozone (Kang et al., 2022a) and it can induce oxidative stress to bacteria, improving the bactericidal effect of PAOA (Theron et al., 2007; Zhou et al., 2020). These results were supported by the disc-diffusion assay (Figure. 9). We found that all PAOA had larger clear zone in both pathogens, compared to organic acids alone. Meanwhile, regardless of organic acid and plasma treatment, *C. jejuni* exhibited a lower microbial reduction compared to *S*. Typhimurium, possibly due to the unique resistance mechanism of *C. jejuni* (Somers et al., 1994). When exposed to an antibacterial agent, *C. jejuni* utilizes its extracellular matrix to form a membrane with a distinct structure, making it difficult to penetrate into the bacterial cell (Somers et al., 1994).

**(A)**

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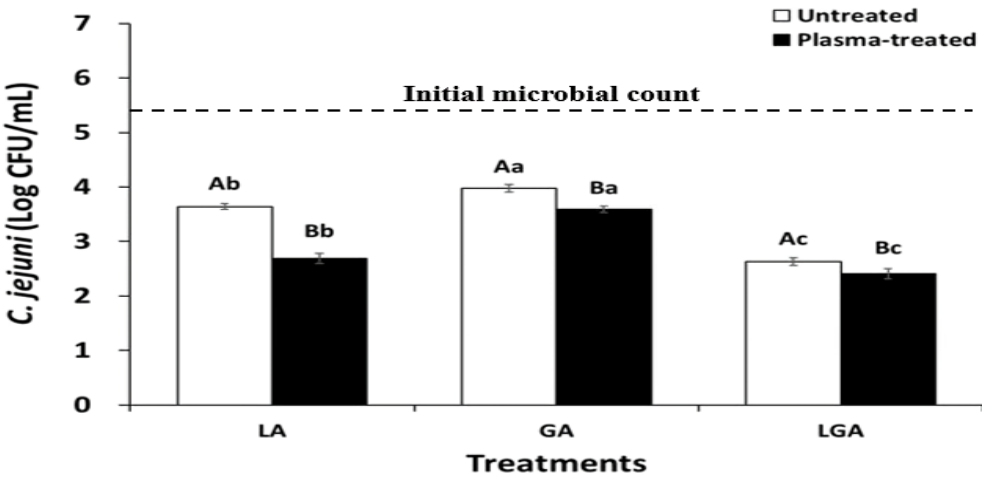



Figure 8. Inactivation effect of plasma-activated organic acids against *Salmonella* Typhimurium (A) and *Campylobacter jejuni* (B). LA, lactic acid; GA, gallic acid; LGA, mixed solution of lactic acid and gallic acid; ND, not detected. [A, B]Different letters indicate significant different ($P < 0.05$) with and without plasma treatment within the same organic acid. [a-c]Different letters indicate significant different ($P < 0.05$) between organic acid treatments or PAOA treatments.

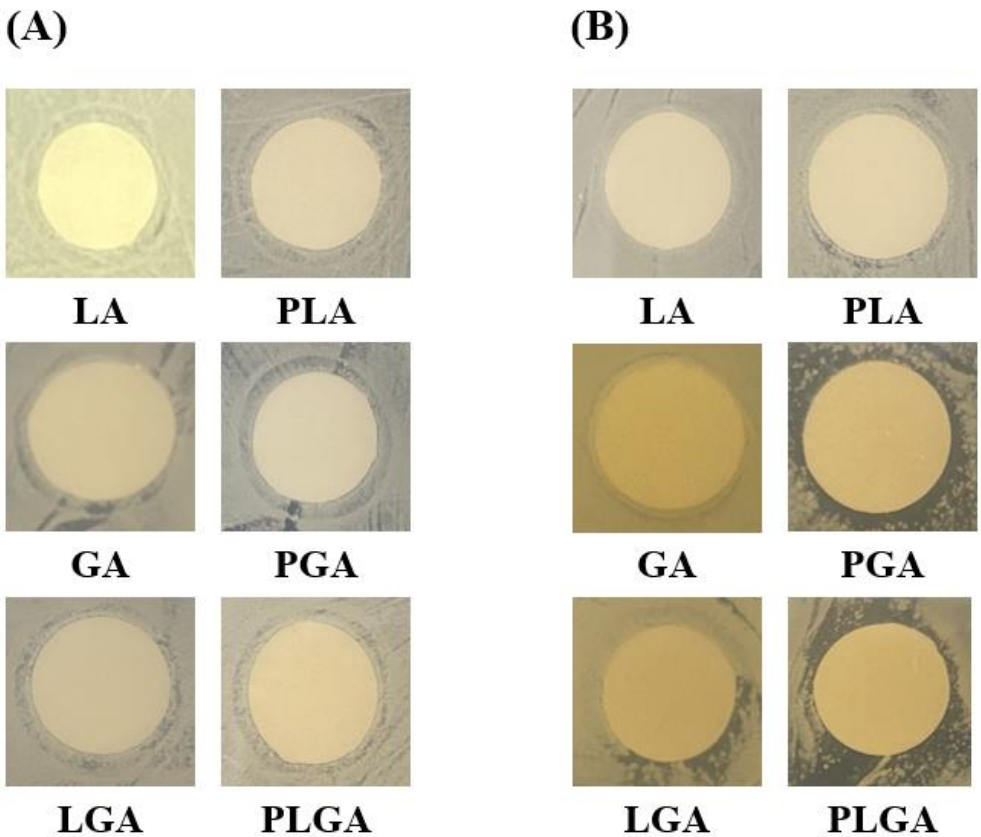



Figure 9. Agar diffusion assay at plasma-activated organic acid against *Salmonella* Typhimurium (A) and *Campylobacter jejuni* (B). LA, lactic acid; GA, gallic acid; LGA, mixed solution of lactic acid and gallic acid; PAL, plasma-activated lactic acid; PGA, plasma- activated gallic acid; PLGA, plasma- activated mixed solution of lactic acid and gallic acid.

### *3.3.1.2 Antioxidant activities*

#### *3.3.1.2.1 Total phenolic contents*

Phenolic content plays a crucial role as an antioxidants activity by engaging in reactions with various free radicals (Aryal et al., 2019). It can contribute to antioxidant activity through the transfer of hydrogen atoms or single electrons, decomposition of peroxides, and chelation of transition metals (Zeb, 2020). In this study, we found no phenolic content in LA, whereas GA and LGA had a significantly higher phenolic content (Table 5). These results can be attributed to the addition of GA, which is a natural polyphenol product (Kim et al., 2006; Jung et al., 2010).

Plasma treatment increased total phenolic contents significantly in organic acids, except for LA (Table 5). In details, total phenolic content in GA and LGA was significantly increased by plasma treatment compared to that in organic acids. This increase in total phenolic content may be attributed to the response of ROS to GA. GA has been reported to induce the polymerization of phenolic compounds by facilitating the formation of carbon-carbon or carbon-oxygen bonds between gallic acid molecules through ROS-induced oxidative stress (Zahrani et al., 2020). This oxidative process also can lead to the production of quinone, which is a type of phenolic compound known for its antioxidant properties (Wang et al., 2019). Furthermore, when GA reacts with hydroxyl radicals, it can form a phenoxyl radical (Strlic et al., 2002). This phenoxyl radical can participate in oxidation-reduction reactions, generating new phenolic compounds and contributing to the overall increase in total phenolic content of GA (Strlic et al., 2002).

*3.3.1.2.2 $ABTS^+$ reducing and DPPH radical scavenging activities*

Regardless of plasma treatment, GA and LGA exhibited the higher $ABTS^+$ reducing and DPPH radical scavenging activities than those in LA ($P < 0.05$) (Table 5). This difference could be induced mainly from addition of GA as it contains the abundant phenolic content. The positive relationship of phenolic contents with $ABTS^+$ reducing and DPPH radical scavenging activities have been reported (Dudonne et al., 2009; Zha et al., 2008), as it can effectively neutralize free radicals and decrease its oxidative stress through direct reaction with free radicals (Jung et al., 2010). Hu, et al. (2016) also stated that the phenolic hydroxyl group in GA can increase its $ABTS^+$ reducing activity by hydrogen and electron donation to free radicals. Furthermore, LA is known for its low antioxidant properties, including both $ABTS^+$ reducing and DPPH radical scavenging activities (Zhang et al., 2019).

When plasma was combined, we expected a synergistic effect on the antioxidant activity of PAOA as plasma treatment increased their phenolic contents (Table 5). However, only DPPH radical scavenging activity was enhanced in PLGA. This could be with different reasons, including phenolic content in LGA. In addition, it was reported that DPPH radical scavenging activity can be increased with plasma treatment due to ROS generation (Ghasempour et al., 2020). PAG was not changed $ABTS^+$ reducing and DPPH radical scavenging activities, however, their values in PAL were even decreased with plasma treatment ($P < 0.05$). Taken together, GA and LGA have excellent antioxidant activity and PLGA, which is the combination of LGA and plasma treatment, had the significantly improved antioxidant activity

among the PAOA.

Table 5. Antioxidant activity of organic acid and plasma-activated organic acid

| Types of acids | Total phenolic contents (mg GAE/mL) | | SEM[1)] | ABTS (mM TE/mL) | | SEM[1)] | DPPH (mM TE/mL) | | SEM[1)] |
|---|---|---|---|---|---|---|---|---|---|
| | None | Treated | | None | Treated | | None | Treated | |
| LA | -[b] | -[c] | 0.0000 | 0.912[Ab] | 0.484[Bb] | 0.0241 | 0.135[Ab] | 0.078[Bc] | 0.0112 |
| GA | 4.284[Ba] | 5.120[Aa] | 0.1098 | 5.455[a] | 5.455[a] | 0.0042 | 0.561[a] | 0.559[b] | 0.0006 |
| LGA | 3.929[Ba] | 4.557[Ab] | 0.0421 | 5.455[a] | 5.453[a] | 0.0013 | 0.563[Ba] | 0.576[Aa] | 0.0011 |
| SEM[2)] | 0.0922 | 0.0268 | | 0.0153 | 0.0128 | | 0.0091 | 0.0013 | |

LA, lactic acid; GA, gallic acid; LGA, mixed solution of lactic acid and gallic acid.

[1)]Standard error of the mean (n=6), [2)](n=9).

[A, B]Different letters indicate significant different ($P < 0.05$) with and without plasma treatment within the same or organic acid.

[a-c]Different letters indicate significant different ($P < 0.05$) between organic acid treatments or PAOA treatments.

### *3.3.2. PAOA on chicken meat*

#### *3.3.2.1 Antibacterial effect*

We applied different organic acids and PAOA to chicken meat (breasts and drumsticks) and analyzed their antibacterial effect during 12 days of storage (Figure. 10 and 11). In chicken breast, the numbers of inoculated *S.* Typhimurium and *C. jejuni* were 5.89 and 6.09 Log CFU/g, respectively (Figure. 10). Immediately after the treatment, all organic acids and PAOA significantly decreased their numbers for both pathogens. Also, their effect was consistently maintained until 6 days. Specifically, LA and LGA exhibited a stronger antibacterial effect for both pathogens than GA, possibly by the addition of LA. This aligns with the results in Figure. 8, suggesting the bactericidal effect of LA, compared to other organic acids (Stanojević-Nikolić et al., 2015). However, PAG and/or PLGA occurred additional decrease with plasma treatment, while PAL was not changed ($P < 0.05$). In addition, PLGA had certain synergistic effect on both *S.* Typhimurium and *C. jejuni* inoculated in chicken breast, regardless of storage days.

For chicken drumsticks, the initial numbers for *S.* Typhimurium and *C. jejuni* were 5.74 and 6.03 Log CFU/g, respectively (Figure. 11). Similar to Figure. 10, the organic acids and PAOA demonstrated bactericidal effects against *S.* Typhimurium and *C. jejuni* inoculated in chicken drumsticks. LA and LGA exhibited a higher bactericidal effect than GA, which was sustained for up to 6 days. With plasma, PAG and PLGA tended to have synergistic bactericidal effect although chicken drumstick has different characteristics from breast. In fact, their effect on chicken breast and

drumstick was relatively lower compared to that on bacterial solution (Figure. 8), possibly by their organic matter (Xiang et al., 2019). The presence of organic matter could reduce ROS concentrations by reacting with bacterial cells and ROS itself (Baek et al., 2020). However, despite of the limitations in chicken meat, their application can still effective for *S*. Typhimurium and *C. jejuni* and these results are comparable to the other studies (Qian et al., 2021; Zhao et al., 2021). Qian et al. (2021) and Zhao et al. (2021) investigated the antibacterial effect of PAL and resulted in a relatively lower effect on chicken drumstick and mackerel, respectively. Therefore, our results show that PAOA, especially the mixture of PLGA, could be potential method for controlling microorganisms in chicken meat as it can promote antibacterial effect not only in its solution but also application for different chicken cuts.

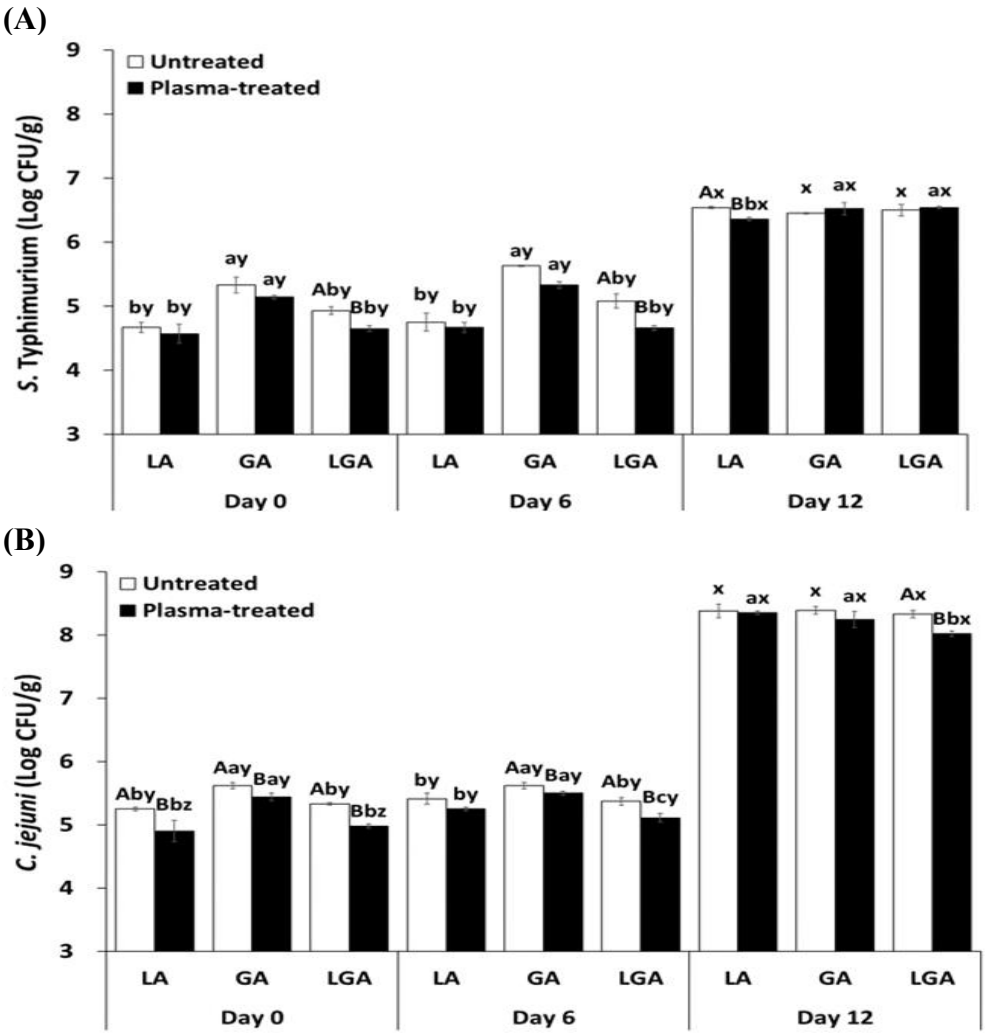
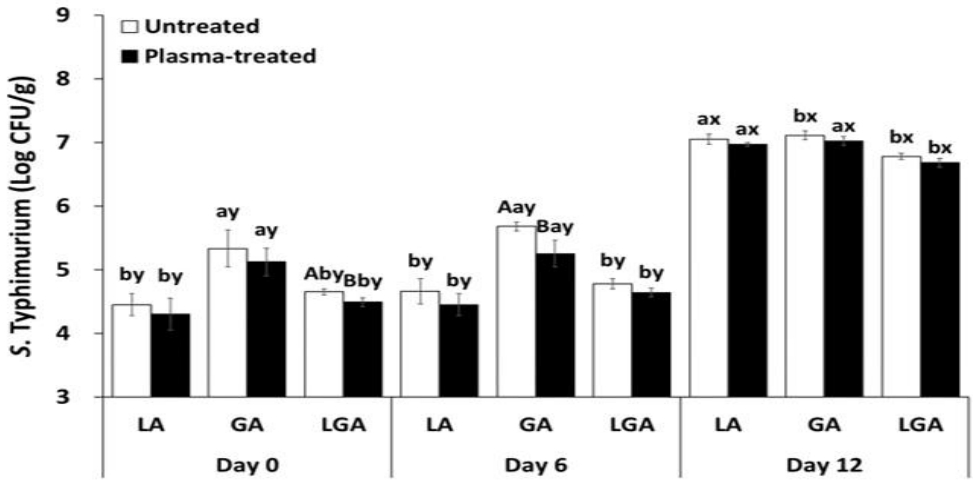



Figure 10. Inactivation of *Salmonella* Typhimurium (A) and *Campylobacter jejuni* (B) inoculated on chicken breast after immersion in plasma-activated organic acids. LA, lactic acid; GA, gallic acid; LGA, mixed solution of lactic acid and gallic acid. [A, B]Different letters indicate significant different ($P < 0.05$) with and without plasma treatment within the same organic acid. [a-c]Different letters indicate significant different ($P < 0.05$) between organic acid treatments or PAOA treatments. [x-z]Different letters indicate significant difference ($P < 0.05$) between different storage days within the same treatment.

**(A)**

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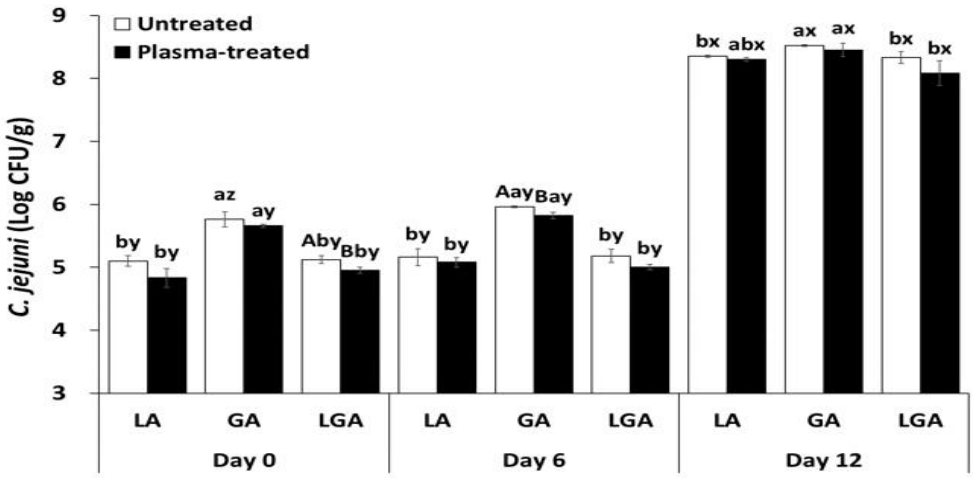


Figure 11. Inactivation of *Salmonella* Typhimurium (A) and *Campylobacter jejuni* (B) inoculated on chicken breast after immersion in plasma-activated organic acids. LA, lactic acid; GA, gallic acid; LGA, mixed solution of lactic acid and gallic acid. [A, B]Different letters indicate significant different ($P < 0.05$) with and without plasma treatment within the same organic acid. [a-c]Different letters indicate significant different ($P < 0.05$) between organic acid treatments or PAOA treatments. [x-

[z]Different letters indicate significant difference ($P < 0.05$) between different storage days within the same treatment.

### *3.3.2.2 Antioxidant activities*

#### *3.3.2.2.1 $ABTS^+$ reducing and DPPH radical scavenging activities*

Similar to the results in Table 5, GA and LGA on chicken breast and drumstick exhibited higher $ABTS^+$ reducing and DPPH radical scavenging activities than LA alone, regardless of different storage days (Tables 6 and 7). In details, both breast and drumstick with GA and LGA had a significantly higher ABTS value compared to LA with/without plasma treatment involved (Table 6), possibly by the higher antioxidant activity in their solution (Table 5). Organic acid itself have antioxidant activity and GA is known for its excellent capacity (He et al., 2020), which explains our findings with the GA and LGA. Their effect can be affected with plasma treatment (Yaru et al., 2020). Here, plasma treatment changed the antioxidant activity of organic acids with different manners during storage days (Table 6). During 12 days of storage, PAL tended to exhibit a lower ABTS value in both chicken cuts than LA, except for chicken breast on days 0 and 12 ($P < 0.05$), while the values in PAG and PLGA was not consistently affected with plasma treatment. Different changes in PAOA could be attributed to the different effect of plasma treatment on different organic acids and this difference may vary lipid oxidation in chicken meat.

Meanwhile, GA and LGA also exhibited higher DPPH radical scavenging activity than LA, regardless of plasma treatment and storage days ($P < 0.05$, Table 7). As shown in Table 5, GA possesses a high level of phenolic content, which contributes to its notable DPPH radical scavenging activity by enhancing the hydrogen ion donating ability of antioxidants (Dudonne et al., 2009). Therefore,

chicken meats treated with GA and LGA generated a greater amount of DPPH-H, resulting in a significantly higher DPPH radical scavenging activity. Our result is accompanied with Limpisophon et al. (2017), who reported the effect of GA on the enhanced DPPH value in fish gelatin film. On the other hand, plasma treatment tended to improve DPPH radical scavenging activity in chicken meat during storage days.

Considering the present results from ABTS and DPPH assays, we found that PAG and PLGA had a certain antioxidant activity and, also, the effect of PLGA was generally maintained during 12 days of storage. Therefore, the use of GA and its mixture, especially PLGA, can inhibit lipid oxidation in different chicken cuts.

Table 6. $ABTS^{+}$ reducing activity (mM TE/g) of chicken meats treated with organic acid and plasma-activated organic acid

| Storage (day) | Organic acids | Breast | | SEM[1)] | Drumstick | | SEM[1)] |
|---|---|---|---|---|---|---|---|
| | | None | Plasma | | None | Plasma | |
| 0 | LA | $1.205^{bx}$ | $1.152^{bx}$ | 0.0142 | $1.253^{Abx}$ | $1.116^{Bbx}$ | 0.0180 |
| | GA | $1.683^{axy}$ | $1.683^{axy}$ | 0.0004 | $1.681^{Bay}$ | $1.683^{Aax}$ | 0.0005 |
| | LGA | $1.681^{a}$ | $1.684^{a}$ | 0.0008 | $1.682^{ay}$ | $1.685^{a}$ | 0.0010S |
| | SEM[2)] | 0.0099 | 0.0062 | | 0.0012 | 0.0147 | |
| 6 | LA | $1.146^{Abxy}$ | $1.055^{Bby}$ | 0.0188 | $1.136^{Aby}$ | $0.944^{Bby}$ | 0.0085 |
| | GA | $1.685^{ax}$ | $1.684^{ax}$ | 0.0008 | $1.684^{axy}$ | $1.683^{ax}$ | 0.0007 |
| | LGA | $1.684^{a}$ | $1.685^{a}$ | 0.0008 | $1.684^{axy}$ | $1.685^{a}$ | 0.0014 |
| | SEM[2)] | 0.0153 | 0.0006 | | 0.0065 | 0.0028 | |
| 12 | LA | $1.094^{by}$ | $1.066^{by}$ | 0.0097 | $1.101^{Abz}$ | $0.923^{Bby}$ | 0.0053 |
| | GA | $1.680^{ay}$ | $1.679^{ay}$ | 0.0017 | $1.685^{Aax}$ | $1.680^{Bay}$ | 0.0008 |
| | LGA | $1.685^{a}$ | $1.682^{a}$ | 0.0016 | $1.688^{ax}$ | $1.688^{a}$ | 0.0003 |
| | SEM[2)] | 0.0066 | 0.0048 | | 0.0032 | 0.0031 | |

LA, lactic acid; GA, gallic acid; LGA, mixed solution of lactic acid and gallic acid.

[1)]Standard error of the mean (n=6), [2)](n=9).

[A, B]Different letters indicate significant different ($P < 0.05$) with and without plasma treatment within the same or organic acid.

[a, b]Different letters indicate significant different ($P < 0.05$) between organic acid treatments or PAOA treatments.

[x-z]Different letters indicate significant difference ($P < 0.05$) between different storage days within the same treatment.

Table 7. DPPH radical scavenging activity (mM TE/g) of chicken meats treated with organic acid and plasma-activated organic acid

| Storage (day) | Organic acids | Breast | | SEM[1)] | Drumstick | | SEM[1)] |
|---|---|---|---|---|---|---|---|
| | | None | Plasma | | None | Plasma | |
| 0 | LA | $0.060^{Bb}$ | $0.078^{Abx}$ | 0.0032 | $0.061^{Bcx}$ | $0.070^{Abx}$ | 0.0005 |
| | GA | $0.181^{Ba}$ | $0.0185^{Aa}$ | 0.0005 | $0.18^{4ax}$ | $0.185^{ax}$ | 0.0003 |
| | LGA | $0.185^{ax}$ | $0.183^{a}$ | 0.0007 | $0.176^{Bb}$ | $0.184^{Aa}$ | 0.0009 |
| | SEM[2)] | 0.0009 | 0.0026 | | 0.0003 | 0.0008 | |
| 6 | LA | $0.059^{Bb}$ | $0.070^{Abx}$ | 0.002 | $0.056^{Bcy}$ | $0.065^{Aby}$ | 0.0005 |
| | GA | $0.181^{Ba}$ | $0.185^{Aa}$ | 0.0007 | $0.180^{Bay}$ | $0.184^{Aay}$ | 0.0002 |
| | LGA | $0.183^{ay}$ | $0.182^{a}$ | 0.0011 | $0.176^{Bb}$ | $0.184^{Aa}$ | 0.0001 |
| | SEM[2)] | 0.0013 | 0.0010 | | 0.0003 | 0.0002 | |
| 12 | LA | $0.055^{b}$ | $0.055^{by}$ | 0.0024 | $0.051^{cz}$ | $0.051^{cz}$ | 0.0010 |
| | GA | $0.180^{Ba}$ | $0.185^{Aa}$ | 0.0002 | $0.180^{Bay}$ | $0.183^{Aaz}$ | 0.002 |
| | LGA | $0.179^{Baz}$ | $0.182^{Aa}$ | 0.0002 | $0.174^{Bb}$ | $0.181^{Ab}$ | 0.0007 |
| | SEM[2)] | 0.0006 | 0.0019 | | 0.0009 | 0.0004 | |

LA, lactic acid; GA, gallic acid; LGA, mixed solution of lactic acid and gallic acid.

[1)]Standard error of the mean (n=6), [2)](n=9).

[A, B]Different letters indicate significant different ($P < 0.05$) with and without plasma treatment within the same or organic acid.

[a-c]Different letters indicate significant different ($P < 0.05$) between organic acid treatments or PAOA treatments.

[x-z]Different letters indicate significant difference ($P < 0.05$) between different storage days within the same treatment.

*3.3.2.2.2 Lipid oxidation*

Excessive lipid oxidation can affect the color, texture, nutrition, and flavor of meat and chicken meat is susceptible to lipid oxidation due to its high polyunsaturated acid content (Kang et al., 2022). In addition, plasma treatment can increase lipid oxidation due to the generation of free radicals as these radicals are the precursors of lipid (Jayasena et al., 2015). Therefore, we measured lipid oxidation in both chicken breast and drumstick during 12 days of storage by malondialdehyde (Table 8). In this study, LA resulted in the highest TBARS value in both cuts, whereas GA and its mixture decreased TBARS value for whole storage period. This may be by the differences in their antioxidant activity shown in Tables 6 and 7. In fact, the effect on GA on inhibiting lipid oxidation has been extensively investigated in previous studies. GA contains high phenolic content and can remove a large amount of oxygen derived free radicals as phenolic compounds can neutralize and scavenge free radicals (Das et al., 2012; Ramli et al., 2020). Also, Luo et al. (2023) reported that lipid oxidation in oyster was decreased with GA due to the antioxidant properties of alkyl esters in GA. Opposite to the effect of GA, LA is known for promoting lipid oxidation as it alters the intracellular oxidation state of lipid substances (Xu, 2009).

When plasma was combined, PAG and PLGA had a significantly lower TBARS value in both chicken cuts compared to that with PAL, except for drumstick on day 0 (Table 8). Meanwhile, plasma treatment did not change TBARS value mostly in chicken breast and drumstick, possibly by the interaction with their antioxidant activity and ROS present in the PAOA solution. However, during storage, PAOA

showed a lower rate of increase in TBARS values compared to each organic acid without plasma treatment. Kang et al. (2022b) also reported that lipid oxidation did not increase when plasma-activated acetic acid was applied to chicken breast and drumsticks. It seems that the effect on PAOA on inhibiting lipid oxidation could be effective for longer period as ROS could be diminished with time (Gao et al., 2022) and only rely on their enhanced antioxidant activity thereafter.

On the other hand, a relatively higher TBARS value in drumstick than breast could be by their different characteristics (e.g. lipid content and fatty acid composition). It is similar to the results of Gong et al. (2010), who reported a higher lipid oxidation in drumstick due to the differences in unsaturated fatty acids and other components. When organic acids were treated on drumstick alone, their lipid oxidation tended to increase with time, however, no significant changes were observed with PAOA. Thus, PAOA may delay oxidation rate in chicken meat especially for drumstick with long storage. Among them, PAG and PLGA had a higher oxidative stability during storage compared to PAL.

Table 8. TBARS value (mg malondialdehyde per kg sample) in chicken meats treated with organic acid and plasma-activated organic acid

| Storage (day) | Organic acids | Breast | | SEM[1)] | Drumstick | | SEM[1)] |
|---|---|---|---|---|---|---|---|
| | | None | Plasma | | None | Plasma | |
| 0 | LA | $0.21^{Baz}$ | $0.25^{Aa}$ | 0.010 | $0.55^{ay}$ | 0.57 | 0.016 |
| | GA | $0.15^{b}$ | $0.15^{b}$ | 0.007 | $0.47^{Bb}$ | $0.53^{A}$ | 0.010 |
| | LGA | $0.17^{aby}$ | $0.15^{by}$ | 0.010 | $0.49^{Bbz}$ | $0.54^{A}$ | 0.011 |
| | SEM[2)] | 0.010 | 0.008 | | 0.010 | 0015 | |
| 6 | LA | $0.29^{Aay}$ | $0.24^{Ba}$ | 0.009 | $0.60^{ax}$ | $0.59^{a}$ | 0.004 |
| | GA | $0.15^{Bc}$ | $0.17^{Ab}$ | 0.004 | $0.51^{b}$ | $0.53^{b}$ | 0.008 |
| | LGA | $0.19^{bxy}$ | $0.18^{bx}$ | 0.008 | $0.53^{by}$ | $0.56^{ab}$ | 0.013 |
| | SEM[2)] | 0.008 | 0.007 | | 0.007 | 0.011 | |
| 12 | LA | $0.29^{Aax}$ | $0.27^{Ba}$ | 0.004 | $0.63^{ax}$ | $0.61^{a}$ | 0.023 |
| | GA | $0.17^{c}$ | $0.17^{b}$ | 0.005 | $0.52^{c}$ | $0.54^{b}$ | 0.009 |
| | LGA | $0.22^{Abx}$ | $0.18^{Bbx}$ | 0.007 | $0.58^{bx}$ | $0.58^{ab}$ | 0.004 |
| | SEM[2)] | 0.007 | 0.004 | | 0.009 | 0.018 | |

LA, lactic acid; GA, gallic acid; LGA, mixed solution of lactic acid and gallic acid.

[1)]Standard error of the mean (n=6), [2)](n=9).

[A, B]Different letters indicate significant different ($P < 0.05$) with and without plasma treatment within the same or organic acid.

[a-c]Different letters indicate significant different ($P < 0.05$) between organic acid treatments or PAOA treatments.

[x-z]Different letters letters indicate significant difference ($P < 0.05$) between different storage days within the same treatment.

## 3.4. Conclusion

All organic acids inactivated *S*. Typhimurium and *C. jejuni* inoculated on chicken meat effectively and their effect was enhanced with plasma treatment. Specifically, PAL and PLGA had a higher effect on antibacterial activity compared to PAG. In addition, chicken meat treated with PAOA inhibited lipid oxidation for both chicken cuts during storage. Within the different PAOA, PAG and PLGA resulted in a higher oxidative stability in chicken breast and drumstick than that with PAL.

Based on these results, PLGA had effective antibacterial effect as well as antioxidant activity. Considering that the primary antibacterial mechanisms of plasma involves the production of reactive species, concerns regarding oxidation are always present when applying plasma technology for food pasteurization. Therefore, we suggest PLGA as a promising method to control microorganisms without adverse effect on different chicken cuts.

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# Summary in Korean

## 돈육 및 계육에 대한 플라즈마 활성 유기산의 살균효과 및 품질 특성 확인

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본 연구에서는 유기물에 대해 제한된 살균력을 갖고 과도한 산화를 유발하는 플라즈마 활성수(Plasma-activated water, PAW)의 한계점을 플라즈마 활성 유기산(Plasma-activated organic acid, PAOA)를 통해 개선하고자 하였다. 실험은 다음과 같이 두 개의 실험으로 진행되었다. 첫번째 실험은 유기물에 대한 PAW의 살균력을 개선하고자 진행되었다. 초산, 구연산, 젖산에 각각 플라즈마를 복합처리하여 생성된 PAOA를 *Salmonella* Typhimurium, *Escherichia coli* O157:H7, *Listeria monocytogenes*가 각각 접종된 돈육의 등심에 처리하였으며, 각 PAOA의 살균력과 돈육 등심의 이화학적 및 관능적 품질을 확인하였다. 두번째 실험은 젖산, 갈산, 젖산과 갈산의 혼합물에 각각 플라즈마를 처리하여 생성된 PAOA를 *S.* Typhimurium과 *C. jejuni*가 각각 접종된 닭 가슴살과 닭 북채에 적용하였

으며, 이때의 각 PAOA의 살균력과 계육의 산화 안정성을 확인하였다.

첫번째 실험에서, 초산, 젖산, 그리고 구연산을 사용한 모든 종류의 PAOA는 PAW 보다 세 가지 병원성 미생물(*S.* Typhimurium, *E. coli* O157:H7, 그리고 *Listeria monocytogenes*)을 효율적으로 불활성화 시켰다. 하지만 병원성 미생물이 접종된 돈육 등심에 대해서는 오직 플라즈마 활성 초산(Plasma-activated acetic acid)만이 PAW 보다 높은 살균 효과를 보였으며 특히, 0.5%의 PAA는 플라즈마 복합처리에 따른 시너지 살균 효과를 가졌다. 이러한 결과는 다른 PAOA에 비해 PAA의 높은 산화 환원 전위와 플라즈마를 복합처리하였을 때 hydrogen peroxide와 ozone과 같은 활성 산소종 농도가 가장 크게 증가한 결과에 기인했을 것이다. 한편, 돈육 등심의 품질에 대해 모든 PAOA는 육색에 부정적인 영향을 미치지 않았으며 pH 또한 정상적인 식육의 범주에 해당하는 결과를 보였다. 반면 돈육 등심의 지질 및 단백질 산화에 대해서 각 PAOA는 다른 결과를 보였다. PAA와 플라즈마 활성 구연산(Plasma-activated citric acid, PAC)은 플라즈마 활성 젖산(Plasma-activated lactic acid, PAL)에 비해 더 낮은 수준의 지질 산화를 보였다. 이는 PAW에 처리된 돈육 등심과 비교했을 때 유의적인 차이를 보이지 않았으며 저장 기간 동안 낮은 수준의 지질 산화를 유지했다. 단백질 산화의 경우 지질 산화 결과와는 다르게 PAA와 PAL에서 우수한 결과를 보였다. PAA와 PAL은 PAW 보다 낮은 carbonyl 함량을 가졌으며, 저장 기간에도 낮은 수준의 단백질 산화를 유지했다. 즉,

PAA는 다른 PAOA에 비해 돈육 등심의 품질에 미치는 부정적인 영향이 가장 적었으며 상대적으로 높은 저장 안정성을 보였다. 하지만 PAA가 처리된 돈육 등심은 다른 처리구들에 비해 높은 감칠맛(umami)과 신맛을 보였는데, 과도한 신맛의 경우 식육의 관능적인 품질에 부정적인 영향을 줄 수 있기 때문에 이에 대한 추가적인 연구가 필요하다.

두번째 실험에서, 젖산, 갈산, 그리고 젖산-갈산 혼합물을 이용한 PAOA는 닭 가슴살과 닭 북채에 접종된 *S.* Typhimurium과 *C. jejuni*를 효율적으로 불활성화 시켰다. 하지만 PAL과 플라즈마 활성 젖산-갈산 혼합물(Plasma-activated mixture of lactic and gallic acid, PLGA)은 플라즈마 활성 갈산(Plasma-activated gallic acid, PAG)보다 높은 살균 효과를 보였으며, 특히 젖산-갈산 혼합물은 다른 유기산에 비해 플라즈마 복합처리 시 높은 시너지 살균 효과를 보였다. 이는 갈산에 비해 더 높은 살균력을 갖는 젖산에 의한 영향이 때문일 것이다. 한편 PAOA는 닭 가슴살과 닭 북채의 지질 산화를 억제하였는데, PAG와 PLGA는 PLA 보다 저장 기간 동안 더 높은 산화 안정성을 보였다. 이것은 갈산의 높은 항산화 활성에 기인했을 것이다. 갈산은 젖산에 비해 높은 총 페놀 화합물 함량과 ABTS 및 DPPH 라디칼 소거능을 갖는다. 특히 젖산-갈산 혼합물은 다른 유기산에 비해 플라즈마와 복합처리 시 항산화 활성이 가장 크게 개선되는 결과를 보였다. 즉, PLGA는 계육에 대해 시너지 살균 효과를 보였을 뿐만 아니라 저장 기간 동안 개선된 산화 안정성을 보여주었다.

본 연구의 결과를 바탕으로 우리는 PAOA가 PAW의 살균력과 산화 안정성을 개선해 줄 수 있는 효율적인 방법이 될 수 있다고 판단한다. 하지만 PAOA의 살균력과 식육에 처리되었을 때 나타나는 항산화 특성에 대한 세부적인 기작에 대해서는 추가적인 연구가 필요하다. 이에 더해 돈육과 계육 이외의 다른 식육에 대한 적용 및 응용이 진행된다면, 식육 산업에서 안전성을 향상시키기 위한 기술로서 PAOA의 활용가치는 증가할 것이라고 사료된다.