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Author	Jiseon Lee1, Sumi Lee1,2, Ui Sub Jung2, Mi-Jung Choi1
Affiliation	1 Department of Food Science and Biotechnology of Animal Resources, Konkuk University, Seoul, Korea 2 Food Research Institute, Ourhome Ltd., Korea
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ORCID (All authors must have ORCID) https://orcid.org	Jiseon Lee (https://orcid.org/0000-0001-9985-6770) Mi-Jung Choi (https://orcid.org/0000-0002-4370-2366)
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CORRESPONDING AUTHOR CONTACT INFORMATION

For the corresponding author (responsible for correspondence, proofreading, and reprints)	Fill in information in each box below
First name, middle initial, last name	Mi-Jung Choi
Email address – this is where your proofs will be sent	choimj@konkuk.ac.kr
Secondary Email address	Konkuk University, 120 Neungdong-ro, Gwangjin-gu, Seoul, Korea
Postal address	05029
Cell phone number	
Office phone number	+82-2-450-3048
Fax number	+82-2-455-3726

Enhancing Prosciutto Characteristics Through Cold Plasma Treatment: From Pathogen Inactivation to Quality Retention

Abstract

This study investigated the efficacy of non-thermal cold plasma treatment in enhancing the safety of prosciutto by inactivating microorganisms while preserving its essential properties. No consistent trends were observed in the prosciutto samples regarding appearance, pH, water content, water-holding capacity, and filter-paper fluid uptake with increasing treatment duration. No significant differences were observed in hardness or water activity among the samples. In contrast, volatile basic nitrogen levels decreased with treatment duration, except at 9 min, while the values of 2-thiobarbituric acid reactive substances increased, likely owing to the inhibition of endogenous enzyme activity and spoilage microorganisms that produce ammonia and trimethylamine. The levels of active hepatitis E virus RNA decreased significantly after 3 min of treatment and were undetectable after 9 min. Additionally, microbial analyses revealed a reduction of approximately 2-log colony-forming units in *Escherichia coli*, *Listeria monocytogenes*, *Salmonella enterica*, *Bacillus cereus*, and *Staphylococcus aureus* after 5 min of treatment ($p < 0.05$). These findings demonstrate that cold plasma treatment is an effective method for microbial inactivation in prosciutto, achieving significant safety improvements while preserving the physical qualities of the product.

Keywords: cold plasma treatment; hepatitis E virus; prosciutto; pathogen; food safety

Introduction

Foodborne viral infections are significant global health issues, with growing understanding over the last several decades (Ranhja et al., 2023). These infections pose health risks and restrict global economic growth (Shirazi et al., 2018). Among foodborne viruses, the hepatitis E virus (HEV) stands out owing to its prevalence in raw and uncooked pork products, which are often associated with human infections (Sasaki et al., 2018). The incidence of HEV in pigs has increased over the last decades. HEV may cause acute hepatitis and can persist in pork meat even after cooking, leading to transmission through contaminated food or water (Ferri and Vergara, 2023). Prosciutto, a traditional dry-cured ham, is known for its delicate texture, distinctive flavor, and extended shelf life. However, it is vulnerable to microbial contamination during production and storage, including from pathogens such as *Staphylococcus aureus* and *Listeria monocytogenes* (Lee et al., 2023). These contaminants pose significant health risks and necessitate effective preservation methods. Traditional thermal pasteurization is unsuitable for prosciutto as it may negatively affect its sensory qualities, including flavor and texture, which are critical for consumer acceptance. Therefore, there is a growing interest in non-thermal pasteurization technologies, such as cold plasma, which can inactivate pathogens while maintaining the desired qualities of the product (Roshanak et al., 2023). This study investigated whether the application of cold plasma technology can ensure the safe consumption of prosciutto without compromising its sensory attributes.

Sterilization processes aim to eliminate pathogens and extend shelf life while preserving nutritional quality. While effective for liquid foods such as milk, traditional heat treatments may not be suitable for solid foods because of the sensitivity of these foods to heat, which can cause undesirable effects (Li et al., 2016). Packaging can reduce the risk of contamination, but surface cross-contamination remains a concern, particularly when pasteurization is applied before packaging (Thirumdas et al., 2015).

In recent years, cold plasma has emerged as a promising non-thermal inactivation method in the food industry (Roshanak et al., 2023). Frequently referred to as the fourth state of matter, plasma is a quasi-neutralized ionized gas that can generate reactive species capable of effectively inactivating pathogens, including bacteria such as *Escherichia coli* and *Bacillus subtilis* and fungi such as *Aspergillus* and *Penicillium* (Mackinder et al., 2020). Cold plasma treatment is particularly advantageous because it operates at low temperatures, avoiding the negative effects associated with heat, such as nutrient degradation and physical changes in foods (Grabowski et al., 2014). Furthermore, the method is energy-efficient, environmentally friendly, and suitable for commercial applications owing to rapid processing and lack of harmful byproducts.

Cold plasma technology has been observed to improve the sensory quality of meat by maintaining color, enhancing texture, and preserving flavor (Hong et al., 2012; Yadav et al., 2019), and it reduces myoglobin oxidation, thereby retaining the fresh red color, and increases tenderness by subtly modifying the structure of surface proteins (Mahnot et al., 2019). The treatment can reduce microbial activity, extending shelf life without adversely affecting sensory attributes. Moreover, it prevents off-flavors resulting from spoilage, thereby ensuring a fresher taste (Astorga et al., 2022). Thus, cold plasma technology enhances meat quality while preserving the natural sensory characteristics of products.

This study evaluated the effectiveness of cold plasma compared to traditional sterilization methods, providing a comprehensive understanding of its potential as a safer and quality-preserving alternative.

Materials and Methods

Materials

Prosciutto and HEV were obtained from OURHOME (Seoul, South Korea) and Chung-Ang University (Seoul, Korea), respectively. Five pathogens—*E. coli*, *L. monocytogenes*, *Bacillus cereus*, *Salmonella enterica*, and *S. aureus*—were diluted in buffered peptone water (Difco, Detroit, MI, USA) before use. All chemicals used in the analysis were of reagent quality and acquired from a local supplier.

Cold Plasma Treatment

A surface dielectric barrier discharge plasma source treated 18 packages. The prosciutto samples (approximately 5 × 9 × 0.15 cm^3, width × length × height) were placed in an acrylic chamber (34 × 45 × 42 cm^3; Plasma Technology Research Center, Gunsan, Korea), in a single layer at regular intervals, and incubated for 0–9 min. Pure nitrogen gas was introduced at 5 L/min. The surface dielectric barrier discharge plasma source was powered by an AC power supply capable of generating an output of up to 15 kV at 25 kHz. The cold plasma treatment was applied for varying durations, ranging from 0 to 9 min, with each treatment performed on a single package at a time to ensure uniform exposure. Five prosciutto slices from each batch were randomly selected for textural analysis, and the remaining slices were used for chemical analysis.

Appearance and Color Analysis

Images of prosciutto samples were captured using a digital camera (α350; Sony, Tokyo, Japan). Using a color reader (CR-10; Konica Minolta Sensing Inc., Tokyo, Japan), the 0lightness (CIE L^*), redness (CIE a^*), and yellowness (CIE b^*) of the samples were measured. Six slices—two from each of the three batches (n = 6, three batches × two replicates)—were used for color measurements. Total color difference (ΔE) was calculated to evaluate the changes between the

color parameters of untreated and treated cold plasma prosciutto (ΔCIE L^*, ΔCIE a^*, and ΔCIE b^*), as shown in equation (1):

$$\Delta E = \sqrt{(\Delta \text{CIE } L^*)^2 + (\Delta \text{CIE } a^*)^2 + (\Delta \text{CIE } b^*)^2} \quad (1)$$

pH Measurements

After homogenizing the prosciutto samples (5 g) with distilled water (45 mL, pH 5.5) for 60 s at 25℃, the pH of the samples was determined using a pH meter (S-220; Mettler Toledo Co., Zurich, Switzerland). Readings for each sample were obtained in triplicate. Before the analysis, the pH meter was calibrated at 25℃ using a standard buffer solution with pH values of 4, 7, and 10. Measurements were performed using six slices (two slices from each of the three batches; n = 6, three batches × 2 replicates).

Estimation of Water Content and Water Activity

The water content in the prosciutto samples was evaluated using the heat-drying method (AOAC, 2012), in which 2-g samples were placed in a dry oven (OF-105; Daihan Scientific Co., Ltd., Gangwon-do, Korea) at 105℃ until a constant weight was reached. The water content was calculated by subtracting the final from the initial weight measurement.

The water activity (a_w) of prosciutto was determined using a water-activity meter (WA-160A; Guangzhou Amittari Instruments Co., Ltd., Guangzhou, China), previously adjusted using a standard solution. The calibration procedure was followed by the method by Stępień et al. (2020) with a slight modification. Four saturated salt solutions (LiCl, $MgCl_2$, NaCl, and KCl) were prepared at 25℃ with reference a_w values of 0.11, 0.33, 0.75, and 0.86, respectively. Water content and a_w were estimated on six sample slices obtained from each of the three batches (n = 6, representing three batches × 2 replicates).

Water Holding Capacity

To measure water holding capacity (WHC), the method of Choi et al. (2018) was used with minor modifications. Briefly, a 1-g sample of prosciutto was centrifuged at 3,000 ×*g* for 10 min at 4℃. WHC was calculated using the following equation (2):

$$\text{WHC (\%)} = (W_2/W_1) \times 100 \quad (2)$$

where, W_1 and W_2 are the sample weights before and after centrifugation, respectively. This measurement was conducted twice per batch (n = 6; three batches × two replicates).

Filter-Paper Fluid Uptake Measurement

Drip loss was measured using the filter-paper fluid uptake method, which was developed by Kaufman et al. (1986). This method is widely used to assess changes in drip loss of pre-packaged and pre-cut pork muscle because it is simple, accurate, and quick. Drip loss of the prosciutto samples due to the plasma treatment was observed. Briefly, the amount of exudate absorbed by the paper was expressed in mg of filter-paper fluid uptake. Before being used to absorb fluids from a prosciutto sample, the filter paper (Whatman #2; diameter, 42.5 mm) was pre-weighed and weighed again (Kauffman et al., 1986).

Texture Profile Analysis

A texture analyzer (CT3; Brookfield Co., USA) was used to measure the adhesiveness and hardness of the samples (Zhou et al., 2019). Samples (1 × 3 × 0.15 cm^3) were collected using a circular plate probe (TA4/1000; 38.1 mm in diameter) and compressed two times to 50% of their initial height at a speed of 1 mm/s and a trigger load of 100 g. For experimental replication, all procedures were repeated five times using a new batch of prosciutto samples (n = 15, three batches × five replicates).

Assessment of 2-Thiobarbituric Acid Reactive Substances

Lipid oxidation was determined from the 2-thiobarbituric acid reactive substances (TBARS) values (Lee et al., 2021). The samples and distilled water were homogenized at a 1:4 ratio. The mixture was filtered, and the filtered sample (0.5 mL) was mixed with 4.5 mL of TBA solution (0.375% TBA reagent, 0.25 N hydrochloric acid, and 15% trichloroacetic acid). Then, the sample combination was heated for 15 min at 95℃ in a water bath (MaXturdy 45; DAIHAN Scientific Co., Ltd., Gangwon-do, Korea), cooled down for 30 min at 25℃, and centrifuged at 3,000 × *g* at 25℃ for 10 min. A spectrophotometer (Multiskan^{TM} GO UV/VIS; Thermo Fisher, Waltham, MA, USA) was used to measure the absorbance of the supernatant at 532 nm. Six slices were used for this evaluation, with two slices from each of the three batches (n = 6, three batches × two replicates).

Volatile Basic Nitrogen Content

Volatile Basic Nitrogen (VBN) content was assessed using the Conway microdiffusion method on six slices—two from each of the three batches (n = 6, two batches × three replicates). After mixing the minced sample with distilled water in a 1:4 ratio, the mixture was allowed to elute for 30 min. A filter paper (Whatman No. 1; GE Healthcare Life Sciences, Sheffield, UK) was used to filter the homogenate. The filtered material (1 mL), 0.01 N H_3BO_3 (1 mL), and Conway solution (1 mL) were added to the outside section of the Conway dish, while a mixture of 0.066% methyl red and 0.066% bromocresol green in aqueous ethanol was added to the inner section. The VBN values were calculated using the following equation (3):

$$\text{VBN (mg\%)} = [(A\text{-}B) \times f \times 0.02 \times 14.007 \times C \times 100]/S \quad (3)$$

where A is the titration volume (mL) of 0.02 N H_2SO_4, B is the titration volume (mL) of the blank, f is the factor of H_2SO_4, S is the weight (g) of the sample, and C is the dilution value.

Preparation and Inoculation of Prosciutto

The samples were prepared according to the method described by Lee et al. (2023) method for testing the effects of cold plasma. The package (20 g) was combined with 1 mL of a 50-fold diluted HEV stock (10^6 plaque-forming units (PFU)/mL) and 100 μL of pathogen (10^3–10^4 colony-forming units (CFU)/mL) and sealed in sterile polyethylene pouches. After drying, the packages were vacuum-packed and subjected to cold plasma treatment for various durations.

Microbiological Analysis

The procedure described by Lee et al. (2023) was used to prepare samples to determine the effects of cold plasma treatment. Each sample was aseptically transferred to a filtered stomacher bag (3M Science, Minneapolis, MN, USA), diluted with an equal amount of saline water, and homogenized for 5 min. The mixture was centrifuged for 30 min at 10,000 ×*g*, followed by centrifugation of the supernatant for 15 min at 8,000 ×*g* (4℃). The QIAamp Viral RNA Mini Kit (QIAGEN, Hilden, Germany) was used to extract RNA from the supernatant according to the manufacturer's instructions.

To measure the inactivation of *L. monocytogenes* and *E. coli*, each 200-mg sample was suspended in 1.8 mL of sterile saline and then diluted multiple times with sterile saline. Next, the samples were homogenized in stomacher bags for 2 min using a stomacher blender (BKST-04C; BioKonvision, Gwacheon, Korea). The samples were spread on selective agar plates and serially diluted after homogenization. Microbiological parameters were estimated in triplicate for each experiment (n = 3, one batch × three replicates).

Statistical Analysis

To test the major effect (cold plasma treatment time), the packages were randomized. A linear mixed model was used to analyze the data collected in this study. The major variables and their interactions were included as fixed effects and random terms of replication (batch) to assess the physicochemical and microbiological parameters. The SPSS software (ver. 24.0, IBM, Chicago, IL, USA) and analysis of variance (ANOVA) were used to assess model significance. Duncan's multiple range test was run as a post-hoc method when results regarding the three principal effects (irradiation time, intensity, and wavelength) were significant ($p < 0.05$).

Results and Discussion

Appearance and Color

The visual appearance of meat is a critical factor that influences consumer acceptance and purchasing decisions (Yadav et al., 2019). Among the attributes that define meat appearance, color plays a pivotal role in shaping consumer preferences (Hong et al., 2012). In this study, the effects of cold plasma treatment on the appearance and color of prosciutto were assessed, and the results are provided in Fig. 1 and Table 1.

Visual observation based on acquired images revealed no substantial changes in the appearance of prosciutto samples treated with cold plasma. However, more detailed analyses of color parameters yielded statistically significant differences regarding the CIE L^* value, which indicates lightness, between the control and cold plasma-treated samples ($p < 0.05$); in particular, a value of 43.25 was recorded for the control and a range of values between 44.96 and 47.45 for the treated samples. This increase in lightness was associated with the duration of plasma exposure, peaking at 7 min and slightly decreasing at 9 min, although this final change was not statistically significant ($p > 0.05$).

The CIE a^* and b^* values of the control (untreated) samples were higher than those of the treated samples, indicating a shift in color due to the cold plasma treatment. This shift can be attributed to the formation of hydrogen peroxide (H_2O_2) and metmyoglobin during cold plasma processing. Cold plasma generates H_2O_2 as one of the reactive oxygen species (ROS). ROS, including H_2O_2, is produced by dissociating water vapor in the atmosphere and subsequent reactions with ionized particles (Misra et al., 2016). Additionally, cold plasma generates nitric oxide (NO) and other reactive nitrogen species (RNS) owing to the interaction of atmospheric nitrogen with the plasma. RNS can react with meat pigments, influencing meat color and contributing to oxidation processes (Fröhling et al., 2012). Both ROS and RNS play important roles in the observed changes in the physicochemical properties of the treated prosciutto. NO reacts with meat pigments such as myoglobin or metmyoglobin to produce NO-myoglobin (bright red) and NO-metmyoglobin (dark brown to red), which are known to improve the red color of meat. However, the relevant content was not determined in this study. This could be related to marinating processes or treatment conditions (Wang et al., 2021). H_2O_2 interacts with myoglobin, leading to oxidation and the formation of metmyoglobin, which is associated with the browning and greenish tint of meat. The oxidation process is likely accelerated with increased processing time, resulting in a higher concentration of metmyoglobin and a corresponding lighter color of meat samples (Fröhling et al., 2012). Other aspects regarding the visual appearance of the prosciutto samples, particularly the green spots observed on the treated surfaces, can be attributed to the generation of reactive species such as H_2O_2 during the cold plasma treatment. ROS can oxidize the porphyrin ring in the heme group of myoglobin, resulting in pigment transformation and the formation of green compounds, such as verdohematin. The discoloration was visually detectable and consistent with the oxidative processes described in previous studies involving plasma treatment (Cheng et al., 2021). Furthermore, the presence of

RNS, including NO, may have contributed to color changes, as they can react with myoglobin to form nitrosylated compounds, which further affect the color characteristics of the meat.

The ΔE values, which represent the overall color difference, ranged between 2.70 and 4.22 across the treatment. According to Jung et al. (2003), a ΔE increase of 10-unit or more is required for a visually perceptible color change in meat. Thus, the relatively low ΔE values observed in this study suggest that the color changes induced by the cold plasma treatment were minimal and likely imperceptible to the naked eye (Fig. 1).

While these findings align with those of Yadav et al. (2019), who reported no significant color changes in cold plasma-treated ham, it is essential to consider the potential implications of even minor color alterations. The slight changes in CIE L^*, a^*, and b^* values, although not visually significant, could influence consumer perception, especially in a commercial setting where product consistency is paramount. Moreover, this study did not assess the long-term stability of these color changes, which could be crucial for the shelf life and marketability of cold plasma-treated prosciutto. In summary, while cold plasma treatment effectively modified certain color parameters, the changes were minor and did not result in noticeable alterations in the appearance of prosciutto.

Moisture-Related Characteristics

The effects of cold plasma treatment on water content, a_w, WHC, and filter-paper fluid uptake of the prosciutto samples are provided in Table 2. These parameters are critical for understanding the impact of cold plasma on moisture dynamics and the quality of meat products. The untreated prosciutto sample had a higher water content value than the cold plasma-treated samples, except for those at 7 min. The water content in the treated samples generally increased with longer treatment duration, except at 9 min, when a slight decrease was observed.

The a_w values of all samples, treated and untreated, did not vary significantly ($p > 0.05$). This indicates that the cold plasma treatment did not substantially alter a_w, implying that the relative humidity or availability of water in the product remained consistent (Pérez-Andrés et al., 2019). The stability of a_w despite changes in water content may be due to the balance between water release from the meat structure and re-absorption into the product, which preserves the overall a_w.

The WHC of untreated prosciutto was the lowest among the samples. This reduction can be attributed to protein unfolding, which occurs due to oxidation induced by the cold plasma treatment. Wang et al. (2021) reported that cold plasma generates reactive species such as ROS and RNS. Interactions between these reactive species and proteins can lead to oxidation. Oxidative stress leads to the formation of cross-links between proteins and water molecules, effectively increasing the WHC of the treated samples (Zhao et al., 2020). No significant differences were observed in WHC among the treated samples, although a slight increase was noted with longer processing times. Filter-paper fluid uptake was used to measure the amount of free or leachable water in the samples; no significant differences were detected between cold plasma-treated prosciutto and the control samples. Among the treated samples, those exposed to cold plasma for 5 min yielded higher values than the other samples. This reduction was closely related to the observed increase in WHC, as more water is retained within the meat structure, thus reducing the amount available for leaching. The exception at 5 min could be due to a transient phase, in which structural changes induced by the plasma treatment temporarily allow more water to be released before stabilization occurs at longer treatment times. According to Warner (2023), moisture in meat muscle typically retains approximately 85% of the water. Cold plasma treatment may induce the loss of interior water by causing protein denaturation through ROS production. Denaturation of surface proteins could create an edible coating layer, resulting in reduced water migration (Chaijan et al., 2022). The slight variations in water content and

holding capacity observed with increased treatment time suggest that cold plasma treatment may induce a gradual but stabilizing effect on the meat microstructure. This effect likely results from oxidative stress, which leads to protein cross-linking and reduced water mobility within the meat matrix (Zhao et al., 2020).

Therefore, cold plasma treatment significantly affects the moisture dynamics of prosciutto, enhancing WHC while reducing the free water content. These changes are achieved without altering a_w, indicating a balanced redistribution of water within the meat. This study highlights the potential of cold plasma as a non-thermal treatment for improving the quality and shelf life of meat products.

Textural and Morphological Properties

Dry-cured ham with desirable texture characteristics typically exhibits intermediate hardness, low bitterness, and low adhesiveness (Zhou et al., 2019). This study focused on measuring hardness and adhesiveness. The time-varying effects of cold plasma treatment on textural parameters of prosciutto are provided in Table 3. In addition, no significant difference in hardness was observed between samples without and with cold plasma treatment ($p > 0.05$). This suggests that cold plasma treatment, under the conditions applied in this study, does not significantly alter the structural integrity of the meat, maintaining a hardness similar to that of the untreated control.

In contrast, a significant decrease in adhesiveness was recorded in the samples treated for 5 min ($p < 0.05$). Despite this decrease, no consistent trend was observed across the different treatment times, indicating that the effect of cold plasma on adhesiveness may be influenced by factors other than treatment duration alone. The lack of a clear pattern suggests that the interaction between plasma-generated reactive species and meat matrix is complex and variable, potentially depending on the specific conditions of each treatment. Moreover, the prosciutto

samples were analyzed within 2 h post-treatment, minimizing the time required for oxidative processes to affect texture. These results suggest that cold plasma treatment effectively preserves the physical characteristics of prosciutto, at least in the short term (Zhao et al., 2020).

The texture characteristics observed in this study align with the results of a scanning electron microscopy (SEM) analysis (Garcia-Gil et al., 2014), which are shown in Fig. 2. SEM helps visualize components of the meat microstructure, such as muscle fibers and connective tissues, which directly influence meat texture and hardness. A more compact and denser microstructure correlates with increased hardness (Chéret et al., 2005). These structural changes can be measured and correlated with TPA results. The SEM images revealed no significant differences in the microstructure of the prosciutto samples, regardless of treatment duration. This microscopic analysis supports the TPA findings, suggesting that cold plasma treatment, as applied here, does not cause substantial alterations to the meat microstructure.

Previous studies by Jayasena et al. (2015) and Kim et al. (2013) reported no significant changes in the texture of various meat cuts, such as pork butts, beef loins, and pork loins, after plasma treatment ($p > 0.05$). These results reinforce the notion that cold plasma treatment is gentle on the physical properties of meat, preserving its textural qualities.

Acidification, Lipid Oxidation, and Protein Degradation Characteristics

The effects of cold plasma treatment on pH, TBARS, and VBN levels in prosciutto were analyzed to assess any potential impact on the quality and shelf life of the product. Changes in the sample pH after cold plasma treatment are provided in Table 4. The pH values of the samples treated for 1, 5, and 9 min were significantly lower than that of the untreated control (at 0 min) ($p < 0.05$). This reduction in pH can be attributed to the formation of acidogenic molecules, particularly nitrogen oxides (NO_x), which are generated during air plasma processing (Kim et al., 2013). NO_x molecules, when dissolved in the aqueous phase of meat, can form nitric and nitrous

acids, leading to a decrease in pH. This phenomenon has been observed in other studies, where the pH of meat and chicken patties treated with cold plasma decreased significantly compared to the values of untreated controls (Gao et al., 2019; Roshanak et al., 2023). The acidification effect of cold plasma is crucial, as it may influence microbial stability and the shelf life of meat.

The TBARS test was conducted to evaluate the extent of lipid oxidation in prosciutto after cold plasma treatment. TBARS values, which indicate the concentration of malondialdehyde (MA), a by-product of lipid peroxidation, were significantly higher in cold plasma-treated samples than in the control ($p < 0.05$). Moreover, the TBARS values increased progressively with treatment duration ($p < 0.05$) but remained below the threshold of 2.5 mg MA/kg in meat, except at the 9-min mark (Roshanak et al., 2023). The observed increase in TBARS values aligned with the findings of Yadav et al. (2019) and Roshanak et al. (2023), who reported that cold plasma treatment of ham, with or without the addition of rosemary extract, resulted in higher lipid oxidation levels. This increase in lipid oxidation is primarily due to the free radicals generated during plasma lipid peroxidation. These free radicals lead to the formation of secondary oxidation products such as alkanes, alkenes, aldehydes, alcohols, ketones, and acids, which further degrade the lipids and proteins in meat (Jayasena et al., 2015).

Additionally, the presence of NO_x, which forms strong complexes with heme pigments during cold plasma processing, inhibits the release of non-heme iron. This inhibition reduces the availability of catalytic iron, which otherwise accelerates lipid oxidation (Astorga et al., 2022). Therefore, although the cold plasma treatment is effectively inactivated, the impact on lipid stability, especially during long-term storage, should be considered.

VBN is a key component of meat freshness, reflecting the degree of protein degradation and bacterial activity (Lee et al., 2023). Table 4 provides the time-varying VBN values of prosciutto subjected to cold plasma treatment. VBN values ranged from 0.88 to 6.24 mg%, with the highest

value observed after just 1 min of treatment. A significant decrease in VBN values was noted as treatment duration increased, except at the 9-min mark.

The observed decline in VBN values suggests that cold plasma treatment inhibits the enzymatic and microbial processes responsible for protein breakdown, thereby reducing the formation of volatile basic nitrogen compounds such as ammonia and trimethylamine. These results are consistent with those of Kim et al. (2024), who reported that while pH did not differ significantly between plasma-treated samples, VBN values decreased with longer plasma exposure. The reduction in VBN is likely due to the inactivation of spoilage microorganisms and endogenous enzymes by the reactive species generated during plasma treatment (Bahmani et al., 2011).

The effectiveness of cold plasma in reducing VBN levels and maintaining meat quality is influenced by several factors, including the type of meat, initial VBN levels, treatment intensity, and duration (Lee et al., 2023). Therefore, further research is necessary to optimize treatment parameters and fully understand the implications of cold plasma on meat quality, particularly over longer storage periods.

Cold plasma treatment significantly affected pH, TBARS, and VBN levels in prosciutto, indicating its potential as a non-thermal method to enhance meat safety and extend shelf life. While this method effectively led to reduced pH and VBN levels, thereby inhibiting microbial growth and protein degradation, it increased lipid oxidation, as evidenced by elevated TBARS values.

Microbial Inactivation

Ensuring food safety depends critically on the effective sterilization of microorganisms. Cold plasma has emerged as a novel and promising technique for food decontamination, offering the potential to sterilize foods while preserving their nutritional value (Ansari et al., 2022). The

inhibitory effects of cold plasma treatment on various pathogens inoculated in prosciutto—HEV, *L. monocytogenes*, *B. cereus*, *S. enterica*, and *S. aureus*—are illustrated in Fig. 3.

The study shows that active HEV RNA and pathogen load in the prosciutto decreased significantly with increasing cold plasma processing time ($p < 0.05$) (Fig. 3A). After 9 min of cold plasma treatment, active HEV RNA was no longer detectable, indicating the high efficacy of cold plasma in inactivating this virus.

The bacterial pathogen test (Fig. 3B–E) revealed a marked reduction in bacteria counts as treatment time increased, but no significant differences were observed in terms of bacterial reduction for treatments longer than 5 min ($p > 0.05$). Overall, compared to the untreated control, cold plasma treatment resulted in a reduction of approximately 2 log CFUs after 9 min, highlighting the substantial antimicrobial effects of the technique.

The enhanced sterilization effect of cold plasma can be attributed to the generation of several reactive species during the process. Nitrite (NO_2^-) produced during plasma processing reacts with H_2O_2 to form peroxynitrite (ONOOH), a potent antimicrobial agent. Additionally, ozone (O_3), another active species commonly produced in air plasma, contributes significantly to the antibacterial effect (Zhao et al., 2020).

Supporting studies by Astorga et al. (2022) and Moutiq et al. (2020) have reported that plasma treatment can generate NO_2^- and nitrate molecules, which are known to inhibit the growth of anaerobic microbes in meat products. This antimicrobial effect is further enhanced by the reduction in pH observed in plasma-treated samples, as lower pH conditions are generally less favorable for bacterial growth.

In conclusion, cold plasma treatment effectively inactivated both viral and bacterial pathogens in prosciutto, providing a potent method for improving food safety without compromising quality. The formation of reactive substances, such as peroxynitrite and O_3, plays a key role in

the observed antimicrobial activity, making cold plasma a valuable tool in the food industry's arsenal to ensure the microbiological safety of meat products.

Conclusion

The findings of this study highlight the effectiveness of cold plasma treatment in enhancing the safety of prosciutto while maintaining its essential physicochemical properties. Treatment led to an increase in water content and a slight decrease in WHC and hardness after 5 min of exposure. The generation of NO_2^- and nitrate molecules during cold plasma treatment was a key factor in the significant reduction in the load of HEV and various bacterial pathogens. These results suggest that cold plasma treatment improves microbial safety in prosciutto while preserving the product's desirable qualities, making it a viable alternative to traditional sterilization. Adopting cold plasma technology in the meat industry could enhance food safety while preserving product quality, offering an effective alternative to traditional sterilization methods.

References

Ansari A, Parmar K, Shah M. 2022. A comprehensive study on decontamination of food-borne microorganisms by cold plasma. Food Chem 4: 100098.

Astorga JB, Hadinoto K, Cullen P, Prescott S, Trujillo FJ. 2022. Effect of plasma activated water on the nutritional composition, storage quality and microbial safety of beef. LWT 154: 112794.

Bahmani ZA, Rezai M, Hosseini SV, Regenstein JM, Böhme K, Alishahi A, Yadollahi F. 2011. Chilled storage of golden gray mullet (*Liza aurata*). LWT 44(9): 1894–1900.

Chaijan M, Chaijan S, Panya A, Nisoa M, Cheong LZ, Panpipat, W. 2022. Combined effects of prior plasma-activated water soaking and whey protein isolate-ginger extract coating on the cold storage stability of Asian sea bass (*Lates calcarifer*) steak. Food Control 135: 108787.

Cheng JH, Chen YQ, Sun DW. 2021. Effects of plasma activated solution on the colour and structure of metmyoglobin and oxymyoglobin. Food Chem 353: 129433.

Chéret R, Chapleau N, Delbarre-Ladrat C, Verrez-Bagnis V, Lamballerie MD. 2005. Effects of high pressure on texture and microstructure of sea bass (*Dicentrarchus labrax L*.) fillets. J Food Sci. 70(8): e477–e483.

Choi MJ, Abduzukhurov T, Park DH, Kim EJ, Hong GP. 2018. Effects of deep freezing temperature for long-term storage on quality characteristics and freshness of lamb meat. Food Sci Anim Resour 38: 959–969.

Ferri G, Vergara A. 2021. Hepatitis E virus in the food of animal origin: a review. Foodborne Pathog Dis 18(6): 368–377.

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

Gao Y, Zhuang H, Yeh HY, Bowker B, Zhang J. 2019. Effect of rosemary extract on microbial growth, pH, color, and lipid oxidation in cold plasma-processed ground chicken patties. Innov Food Sci Emerg Technol 57: 102168.

Garcia-Gil N, Santos-Garcés E, Fulladosa E, Laverse J, Nobile MAD, Gou P. 2014. High pressure induces changes in texture and microstructure of muscles in dry-cured hams. Innov Food Sci Emerg Technol 22: 63–69.

Grabowski M, Strzelczak A, Dąbrowski W. 2014. Low pressure cold plasma as an alternative method for black pepper sterilization. J Life Sci 8:931–939.

Hong GP, Chun JY, Lee SK., Choi MJ. 2012. Effects of non-meat protein binders and acidification on the efficiency of cold-set pork restructuring by high pressure. Food Sci Anim Resour 32: 301–307.

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

Jung S, Ghoul, M, de Lamballerie-Anton M. 2003. Influence of high pressure on the color and microbial quality of beef meat. LWT 36: 625–663.

Kauffman RG, Eikelenboom G, Van der Wal PG, Merkus G, Zaar M. 1986. The use of filter paper to estimate drip loss of porcine musculature. Meat science, 18(3): 191–200.

Kim HJ, Yong HI, Park S, Choe W, Jo C. 2013. Effects of dielectric barrier discharge plasma on pathogen inactivation and the physicochemical and sensory characteristics of pork loin. Curr Appl Phys 13(7): 1420–1425.

Kim SH, Roy PK, Jeon EB, Park SY. 2024. The efficacy of floating electrode-dielectric barrier discharge plasma against *Staphylococcus aureus* and *Salmonella typhimurium* on fried fish paste. Appl Sci 14(5): 2076–3417.

Lee S, Kim EJ, Park DH, Choi MJ. 2021. Two-stage air thawing as an effective method for controlling thawing temperature and improving the freshness of frozen pork loin. LWT 140: 110668.

Lee S, Lee J, Shin J, Choi MJ. 2023. Effects of high-pressure processing on the physicochemical properties of prosciutto and microbe inactivation. LWT 187: 115294.

Lee J, Lee S, Jung US, Lee S, Hong GP, Choi MJ. 2024. Effect of UV-C irradiation on foodborne pathogens in prosciutto and changes in physicochemical properties. Food Sci Anim Resours.

Li L, Li J, Shen M, Hou J, Shao H, Dong Y, Jiang J. 2016. Improving seed germination and peanut yields by cold plasma treatment. Plasma Sci Technol 18(10): 1027.

Mackinder MA, Wang K, Zheng B, Shrestha M, Fan QH. 2020. Magnetic field enhanced cold plasma sterilization. Clin Plasma Med 17:100092.

Mahnot NK, Mahanta CL, Farkas BE, Keener KM, Misra NN. 2019. Atmospheric cold plasma inactivation of Escherichia coli and Listeria monocytogenes in tender coconut water: Inoculation and accelerated shelf-life studies. Food Control 106: 106678.

Moutiq R, Misra NN, Mendonça A, Keener K. 2020. In-package decontamination of chicken breast using cold plasma technology: Microbial, quality and storage studies. Meat Sci 159: 107942.

Pérez-Andrés JM, Á lvarez C, Cullen PJ, Tiwari BK. 2019. Effect of cold plasma on the techno-functional properties of animal protein food ingredients. Innov Food Sci Emerg Technol 58: 102205.

Ranjha MMAN, Shafique B, Aadil RM, Manzoor MF, Cheng JH. 2023. Modification in cellulose films through ascent cold plasma treatment and polymerization for food products packaging. Trends in Food Sci Technol 134: 162–176.

Roshanak S, Maleki M, Sani MA, Tavassoli M, Pirkhezranian Z, Shahidi F. 2023. The impact of cold plasma innovative technology on quality and safety of refrigerated hamburger: Analysis of microbial safety and physicochemical properties. Int J Food Microbiol 388: 110066.

Sasaki Y, Haruna M, Uema M, Noda M, Yamada Y. 2018. Prevalence and phylogenetic analysis of hepatitis E virus among Pigs in Japan. Jpn J Infect Dis 71:75–78.

Shirazi R, Pozzi P, Wax M, Bar-Or I, Asulin E, Lusting Y, Mendelson E, Ben-Ari Z, Schwartz E, Mor O. 2018. Hepatitis E in pigs in Israel: Seroprevalence, molecular characterization and potential impact on humans. Euro Surveill 23:1800067.

Stępień A, Witczak M, Witczak T. 2020. Moisture sorption characteristics of food powders containing freeze dried avocado, maltodextrin and inulin. Int J Biol Macromol 149: 256-261.

Thirumdas R, Sarangapani C, Annapure US. 2015. Cold plasma: a novel non-thermal technology for food processing. Food biophys 10:1–11.

Wang X, Wang Z, Zhuang H, Nasiru MM, Yuan Y, Zhang J, Yan W. 2021. Changes in color, myoglobin, and lipid oxidation in beef patties treated by dielectric barrier discharge cold plasma during storage. Meat Sci 176: 108456.

Warner RD. 2023. The eating quality of meat: IV—Water holding capacity and juiciness. In Lawrie's meat science (pp. 457–508). Woodhead Publishing.

Yadav B, Spinelli AC, Govindan BN, Tsui YY, McMullen LM, Roopesh MS. 2019. Cold plasma treatment of ready-to-eat ham: Influence of process conditions and storage on inactivation of *Listeria innocua*. Food Res Int 123: 276–285.

Zhao Y, Chen R, Tian E, Liu D, Niu J, Wang W, Qi Z, Xia Y, Song Y, Zhao Z. 2018. Plasma-activated water treatment of fresh beef: Bacterial inactivation and effects on quality attributes. IEEE Trans Radiat Plasma Med Sci 4(1): 113–120.

Zhou CY, Wang C, Cai JH, Bai Y, Yu XB, Li CB, Xu XL, Zhou GH, Cao JX. 2019. Evaluating the effect of protein modifications and water distribution on bitterness and adhesiveness of Jinhua ham. Food Chem 293: 103-111.

Table 1
Color measurements of prosciutto treated to cold plasma.

Treated time (min)	L*	a*	b*	ΔE
0	43.25±2.37^{c}	9.91±0.95^{a}	8.16±1.23^{a}	-
1	44.96±0.53^{b}	7.92±0.02^{c}	7.64±0.24^{a}	2.70±0.40^{b}
3	45.99±0.23^{b}	9.73±0.34^{a}	8.05±0.42^{a}	2.79±0.24^{a}
5	46.79±0.72^{a}	9.60±0.52^{a}	8.04±0.23^{a}	3.07±0.14^{a}
7	47.45±0.05^{a}	9.26±0.45^{ab}	8.23±0.11^{a}	4.22±0.09^{a}
9	46.46±0.04^{a}	8.64±0.10^{bc}	8.41±0.10^{a}	3.46±0.04^{a}

$^{a\text{-}c}$Means with different superscript letters in a column are significantly different ($P < 0.05$).

Table 2
Water-related characteristics of prosciutto treated to cold plasma.

Treated time (min)	Water content (%)	Water activity	WHC (%)	FFU (mg)
0	48.18±0.29^{ab}	0.78±0.00^{a}	98.32±0.50^{c}	4.80±0.34^{ab}
1	46.65±2.11^{c}	0.78±0.00^{a}	98.34±0.08^{c}	3.44±0.12^{b}
3	46.37±1.39^{c}	0.78±0.00^{a}	98.71±0.19^{abc}	4.22±0.33^{ab}
5	47.48±0.47^{bc}	0.78±0.00^{a}	98.40±0.24^{bc}	5.52±0.31^{a}
7	49.03±0.61^{a}	0.78±0.00^{a}	98.07±0.02^{a}	3.15±0.17^{b}
9	47.01±1.85^{bc}	0.78±0.00^{a}	98.77±0.09^{ab}	3.50±0.23^{b}

WHC, water holding capacity; FFU, filter-paper fluid uptake.
$^{a-c}$Means with different superscript letters in a column are significantly different ($p<0.05$).

Table 3
Texture profile analysis of prosciutto treated to cold plasma.

Treated time (min)	Hardness (g)	Adhesiveness
0	5024.2±304.8[a]	0.6±0.1[a]
1	5033.8±502.3[a]	0.6±0.2[a]
3	5091.3±464.7[a]	0.7±0.1[a]
5	5046.8±861.1[a]	0.5±0.2[a]
7	5049.3±478.7[a]	0.6±0.2[a]
9	5029.9±784.4[a]	0.7±0.1[a]

[a]Means with different superscript letters in a column are significantly different ($p<0.05$).

Table 4
pH, TBARS, and VBN measurements of prosciutto treated to cold plasma.

Treated time (min)	pH	TBARS (mg MA/kg)	VBN (mg%)
0	6.03±0.06^{c}	0.33±0.02^{f}	3.67±0.00^{b}
1	6.00±0.01^{d}	0.58±0.02^{e}	6.24±4.04^{a}
3	6.06±0.01^{b}	0.80±0.03^{d}	3.21±4.04^{b}
5	6.00±0.01^{d}	1.09±0.02^{c}	2.27±0.00^{c}
7	6.12±0.02^{a}	2.08±0.04^{b}	0.88±0.00^{d}
9	5.92±0.02^{e}	3.82±0.08^{a}	3.44±4.04^{b}

TBARS, thiobarburic acid reactive substance; VBN, volatile basic nitrogen; MA, malonaldehyde.
$^{a\text{-}f}$Means with different superscript letters in a column are significantly different ($P < 0.05$).

Figure captions

Fig. 1. Appearance of prosciutto; samples after application of cold plasma treatment for 0 (A), 1 (B), 3 (C), 5 (D), 7 (E), and 9 min (F).

Fig. 2. Scanning electron microscopy images of prosciutto. The samples after plasma treatment for 0 (A), 1 (B), 3 (C), 5 (D), 7 (E), and 9 min (F). The scale bar is 100 µm.

Fig. 3. Total and active RNA of HEV (A), and inactivation of *L. monocytogenes* (B), *B. cereus* (C), *S. enterica* (D), and *S. aureus* (E) of prosciutto treated with cold plasma. $^{a\text{-}d}$ Means with different letters are significantly different ($p<0.05$).

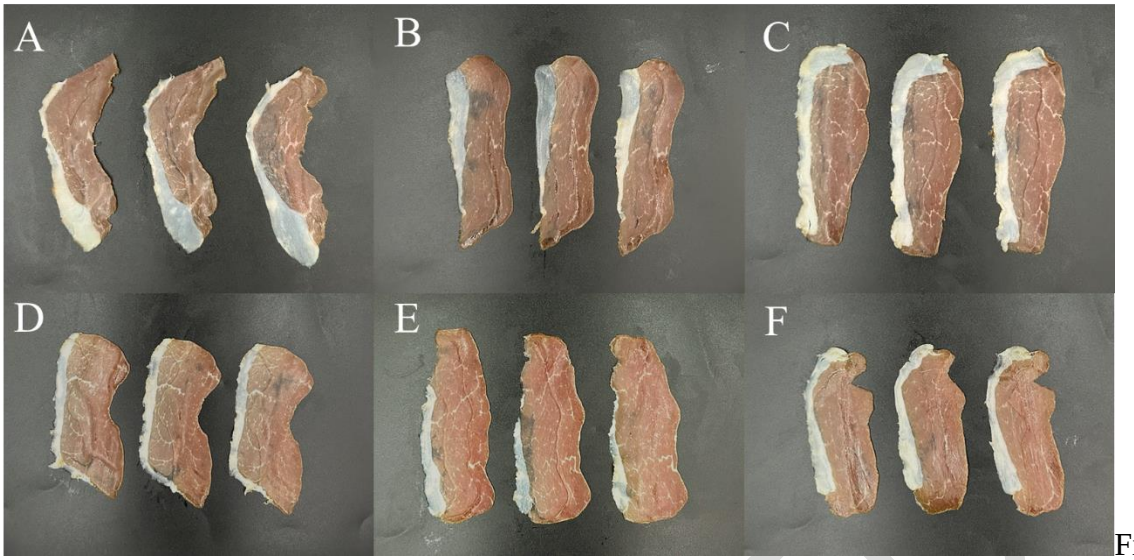


Figure 1.

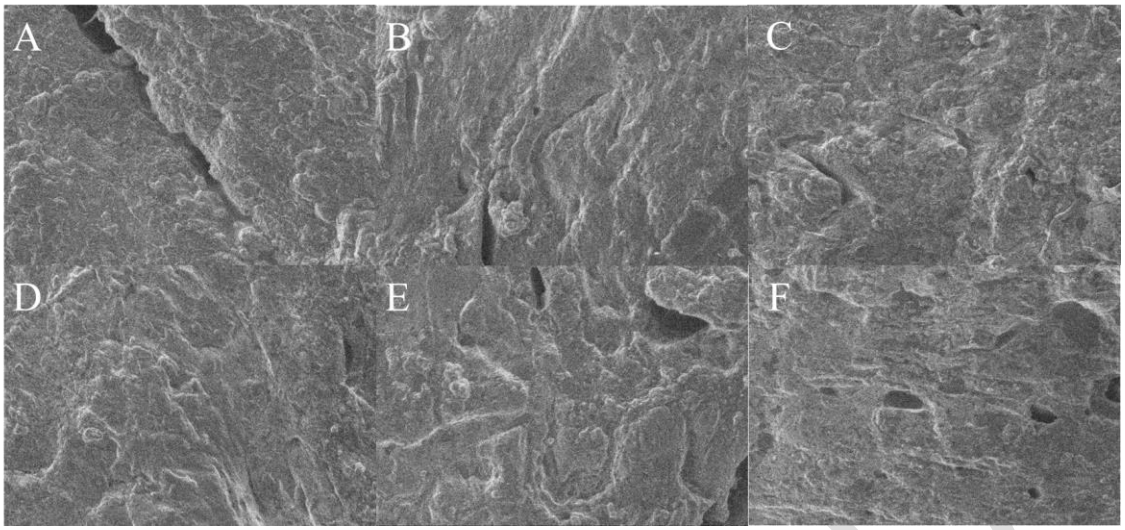
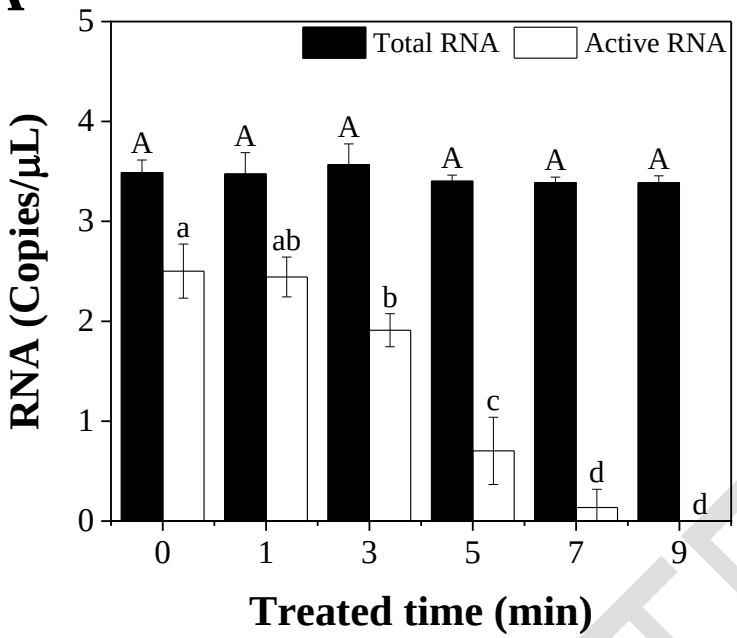
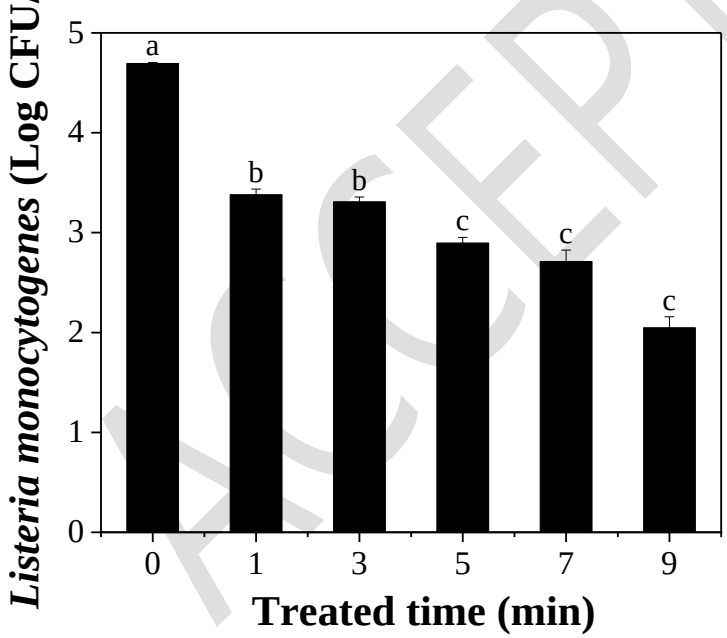


Figure 2.

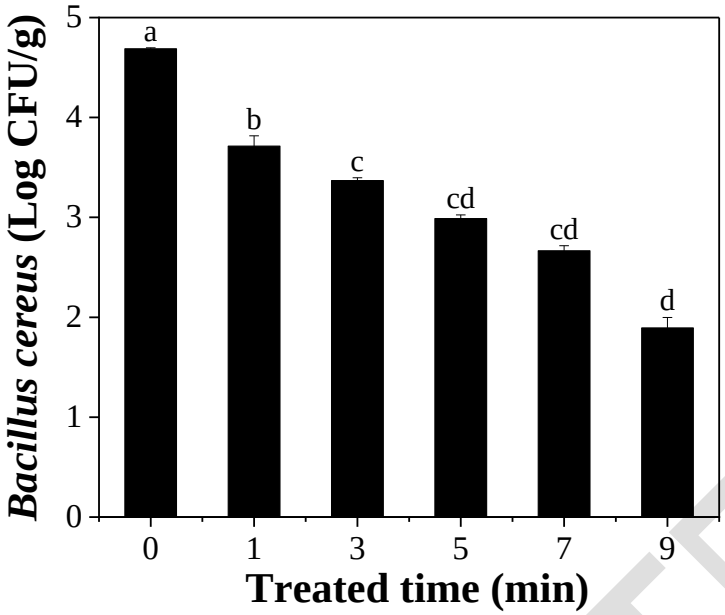
A


Total RNA
Active RNA
RNA (Copies/μL)
Treated time (min)
A
A
A
A
A
A
a
ab
b
c
d
d
0
1
3
5
7
9

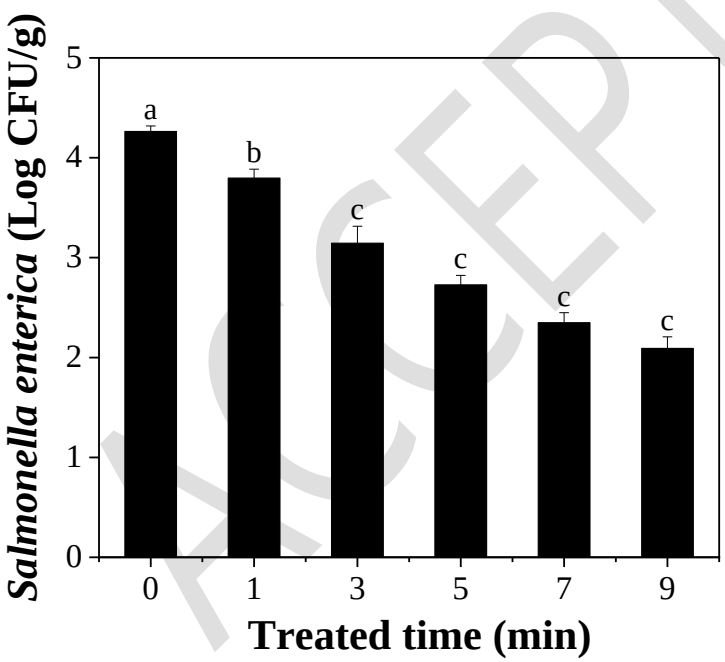
B


Listeria monocytogenes (Log CFU/g)
Treated time (min)
a
b
b
c
c
c
0
1
3
5
7
9

C


Bacillus cereus (Log CFU/g)
0
1
2
3
4
5
a
b
c
cd
cd
d
0
1
3
5
7
9
Treated time (min)

D


Salmonella enterica (Log CFU/g)
0
1
2
3
4
5
a
b
c
c
c
c
0
1
3
5
7
9
Treated time (min)

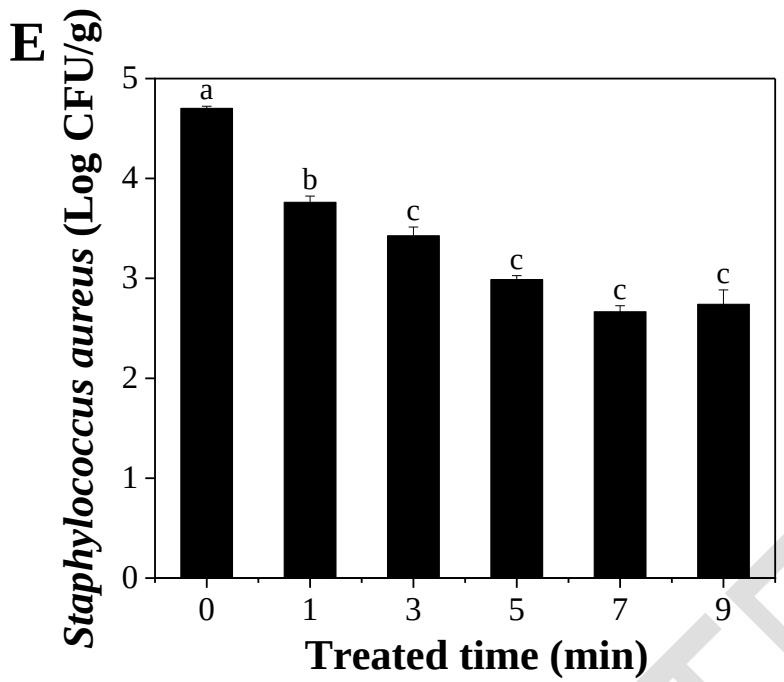


Figure 3.