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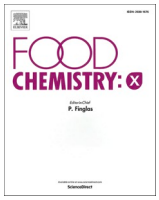

Combating foodborne pathogens: Efficacy of plasma-activated water with supplementary methods for *Staphylococcus aureus* eradication on chicken, and beef

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ABSTRACT

The research study suggested using plasma-activated water (PAW) along with auxiliary technologies, such as micro/nanobubbles (MNB), ultraviolet (UV) photolysis, and ultrasonication (US), to increase the effectiveness of sterilization. By using Factorial Design of Experiments (DOE) techniques, the characteristics and optimal production that contributed to disinfecting pathogens were assessed. Analysis revealed that *Staphylococcus aureus* (*S. aureus*) infection rate was most significantly influenced by factors including duration of MNB, UV, and the interaction term between MNB*UV. The optimal conditions for *S. aureus* reduction in chicken and beef of 8.41 and 8.20 $\log_{10}$ CFU/ml, respectively, which were found when PAW was combined with UV and US for 20 min of treatment. This study arrives to the conclusion that combining PAW with appropriate supplementary technologies increased efficiency and enhance disinfection effectiveness in chicken and beef which could be implemented for another alternative pathogen inactivation in food industry.

1. Introduction

Food contamination from biological and chemical sources is currently leads to foodborne diseases (Cooper et al., 2015; Cunault et al., 2018). A total of 962 foodborne disease outbreaks (FBDOs) were recorded, leading to over 8000 illnesses, over 1000 hospitalizations, and 20 deaths. Bacterial infections were the primary cause of both outbreaks and illnesses, while poisonous mushrooms were the leading cause of fatalities. Most outbreaks occurred in homes, followed by restaurants and canteens. Animal-based foods were the most common culprit in FBDOs, followed by poisonous mushrooms and plant-based foods. Poisonous mushrooms were particularly problematic in homes, whereas bacteria were the main issue in other settings. *Vibrio parahaemolyticus* was the most frequent bacterial cause and was primarily linked to seafood. Different types of bacteria were associated with different food groups (Chen et al., 2022). 48 million Americans, according to the Centers for Disease Control and Prevention, have had a foodborne illness, which causes 128,000 hospitalizations and around 3000 fatalities annually (Johnson et al., 2014). Food safety management aims to eliminate or significantly reduce harmful bacteria in food. Early detection of these pathogens is crucial to prevent widespread foodborne illnesses (Pinu, 2016). The widespread use of antibiotics in animal

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agriculture since the 1930s has contributed to the development of antibiotic-resistant bacteria. This has led to the emergence of new foodborne pathogens since 2000, including Campylobacter, Norovirus, Salmonella, and Listeria (Allen, 2014). Food poisoning caused by Staphylococcus bacteria has been a persistent problem in the milk and meat industries since 1894 (Jakobsen et al., 2011). In 2015, the EU reported a significant number of foodborne outbreaks primarily caused by Salmonella and Campylobacter. Other pathogens, toxins, viruses, and parasites also contributed to these outbreaks (Vidyadharani et al., 2022). Future foodborne illness outbreaks are anticipated to increase as a result of increased business activity, a growing global population, and the effects of environmental deterioration. Over time, these issues have been resolved using a variety of pertinent technologies. Technologies that are nonthermal have been studied as a substitute platform to reduce food-borne microbial hazards (He & Shi, 2021).

Red meat is a significant nutritional resource for humans and a key contributor to national economies. However, the industry is under scrutiny due to its association with widespread public health concerns. Specifically, foodborne illnesses linked to red meat and its products have caused numerous outbreaks with detrimental impacts on individuals, businesses, and society. This study systematically reviews three decades of published investigations into global foodborne disease outbreaks attributed to red meat consumption (Warmate & Onarinde, 2023). Food safety incidents within the red meat sector represent a significant global public health challenge (Shang & Tonsor, 2017). Contamination, frequently occurring during post-harvest stages such as processing, distribution, or retail (Food Standards Agency et al., 2020), can trigger foodborne disease outbreaks. These incidents can necessitate product recalls, enforcement actions against implicated businesses, and may result in substantial economic losses and human casualties (Hussain & Dawson, 2013; Robertson et al., 2016). Microbial contaminants, particularly bacterial pathogens, are primary contributors to severe food safety issues in the red meat supply chain (Sofos, 2008). While various food sources can transmit illnesses, meat and its derivatives are prominent vectors for human infections (Nørrung et al., 2009). Numerous studies have identified red meat, including beef and pork, as a causal factor in foodborne disease outbreaks (Bélanger et al., 2015; Bryan, 1980; Jeffer et al., 2021; Omer et al., 2018).

Over the past few decades, plasma technology has garnered significant interest in food processing applications. A particularly attractive characteristic of plasma is its ability to achieve potent microbial inactivation at low temperatures. This characteristic makes it suitable for food products that are sensitive to heat-based treatments. Plasma treatments can be employed in food processing for various purposes, including surface decontamination (leaching), modification of surface properties, and enhancement of mass transfer on diverse food surfaces. These capabilities offer distinct advantages for the food industry.

Non-thermal plasma (NTP) treatment of water samples leads to the generation of Plasma-Activated Water (PAW) through interactions with reactive species produced by the NTP. These interactions include the formation of free radicals, documented in previous studies (Ma et al., 2015; Shen et al., 2016). PAW is consequently enriched with a complex mixture of short-lived reactive oxygen and nitrogen species (ROS and RNS), collectively termed reactive oxygen and nitrogen species (RONS). The application of electric gas discharges in air or nitrogen-oxygen mixtures across a volume of water occurs before PAW synthesis. The emphasis of the study is the visible liquid phase plasma chemistry, which is produced by straightforward atomic and molecular precursors, as described in the section titled “Plasma Fundamental Considerations”. One of the species implicated is RNS based on nitrogen oxides. (NO, NO_2, NO_3, N_2O_3, N_2O_4, N_2O_5) nitrogen oxoacids based RNS ($H_aN_bO_c$) and ROS (O_3, O_2, O, OH, HO_2, H_2O_2). PAW solutions typically comprise nitric (HNO_3) and nitrous (HNO_2) acid and low level transient RNS like e.g. peroxynitrous. Acid ONOOH / peroxynitrite $ONOO^-$ and accompanying ROS. Plasma is infused into distilled water for a predetermined amount of time to produce PAW. As an aqueous disinfectant, PAW has drawn a lot of interest (Shen et al., 2016). Plasma treatment of water can yield chemical substances such as hydrogen peroxide (H_2O_2), nitrite (NO_2^-), and nitrate (NO_3^-). Additionally, particular interactions between plasma-derived species and water may produce both long-lasting nitric acid and transitory reactive species including OOH, OH, and H radicals (Xu et al., 2017; Zhou et al., 2018). The synergistic effects of a strong positive oxidation reduction potential (ORP) and a low pH, both caused by the substances mentioned above, are what give PAW its bactericidal activity (Liao et al., 2020; Zhang, Sun, et al., 2016). *Escherichia coli*, *Hafnia alvei*, *Pseudomonas deceptions*, and *S. aureus* are just a few of the bacteria that PAW can effectively inactivate (Brisset et al., 2008; Traylor et al., 2011).

According to the earlier research, PAW has been used to effectively inactivate food-borne viruses in chicken flesh. However, the efficiency of the disinfection procedure is diminished when the meat becomes thicker and contains a significant amount of fat layer. This study proposes a novel sterilization technique for chicken meat. It investigates the synergistic application of PAW, MNB and US to enhance pathogen inactivation efficacy against *S. aureus*. Furthermore, the research aims to optimize production parameters associated with *S. aureus* inactivation. In addition, optimal conditions are applied to other types of meat such as beef. Factorial design was employed for planning and analyzing the optimal conditions.

2. Materials and methods

In order to boost sterilizing efficacy, the research study recommended combining PAW with supplementary technologies such MNB, UV and US that process of experiment.

2.1. PAW combined with supplementary disinfection technique

To effectively decrease common food-borne pathogens using Plasma Technology and supplementary disinfection techniques (UV, US), The reseacher need a strong foundation. This includes a theoretical understanding of these technologies and the pathogens target. Additionally, reviewing existing research is crucial to identify significant problems and best practices. This review should encompass common food-borne pathogens, the principles of plasma technology and supplementary disinfection techniques.

In the study of the design experiment and analysis of plasma factors using a factorial experiment design technique at the center (2^k factorial design with center point) to determine the factors in UV 0–20 min, US 0–20 min. It has a boundary level of high, low, and midpoint levels. Depending on the circumstances that the experiment was intended to assess the impact of plasma on the reduction of the amount of common food-borne pathogens in chicken meat. The response is the amount of common food-borne pathogens and quality of chicken and beef. The target value of which is the decrease of common food-borne pathogens in poultry industry. Determine the factors by optimal condition of supplementary disinfection techniques when combining with PAW for the reduction of food-borne pathogens from previous experiment (Moonsub et al., 2024). Factor of PAW combined with UV, US and UV*US process. To optimal condition of supplementary disinfection techniques when

Table 1
Design experiment of the PAW combined with supplementary disinfection technique in food-borne pathogen.

Symbol	Factors	Unit	Boundary Level		Reference
			Minimum (−1)	Maximum (+1)	
Factor1	**Ultraviolet Radiation Time**	**min**	**0**	**20**	**(Katara et al., 2008)**
Factor2	**Ultrasonication Time**	**min**	**0**	**20**	**(Royintarat et al., 2020)**

combining with PAW for the reduction of food-borne pathogens during process. The factors and levels of experiment shown in Table 1. Design experiment of the PAW combined with supplementary disinfection technique in food-borne pathogens during process of chicken meat experiment shown in Table 2.

2.2. Bacteria culture

An overnight starter culture of *S. aureus* was prepared by aseptically transferring a loopful of culture from a fresh slant to a flask containing 150 ml of Nutrient Broth (NB). The culture was incubated at 37 °C with shaking at 150 rpm for 24 h. Subsequently, 10 ml of fresh NB was aseptically transferred to a new culture flask. Both the starter culture and the new flask were incubated at 37 °C with shaking at 150 rpm for an additional 24 h. Spectrophotometric analysis at an optical density (OD) of 600 nm was used to estimate the concentration of *S. aureus* cells in a 10 ml diluted sample. An OD reading of 1.0 corresponded to an estimated concentration of 8×10^8 cells/ml.

2.3. Experimental set up of PAW combining with MNB UV US and Paw'ggeneration

The experiment used the PAW system with Flyback Transformer (FBT). In the previous study, this PAW system can produce H_2O_2 concentration values between 0.5 and 1.68 ppm with efficiently inactive food-borne pathogens (Royintarat et al., 2019).The experiment used a single electrode setup to create underwater plasma without significantly heating it (non-thermal). This underwater plasma was generated using a constant electrical current (DC power supply).

This setup uses a single copper wire electrode encased in a quartz tube. Stainless steel serves as the ground. The system is powered by a 30-watt FBT for 20 min at room temperature (25 °C). With the electrode just 5 mm from the ground (beneath the water surface), the device creates a plasma discharge. The range of the operating condition of the supplementary techniques were identified based on the previous study. For instance, the setup of an in-house MNB system was set at a pressure of 3 bar for 10 min at 25 °C (Moonsub et al., 2024). In the meantime, the UVC lamp (OZUAR, China) was installed and operated at 9-W, UV with wavelength 253.7 nm (Bacteria disinfection 100-280 nm). The US Digital Ultrasonic Cleaner Model 20 A, Motor Conductor performed with 300 mm, Ultrasonic power 120-W, Volt AC220-240 V, Frequency 40 kHz (Bacteria disinfection 24–80 kHz (Maher et al., 2020)), 25 °C operating temperature, Full Capacity 3 L (OEM, Thailand). The 400 ml of tap water has been used in the experiment. The plasma production process begins with the use of MNB before PAW, UV and US production (PAW' Condition).

Table 2
Survival of *S. aureus* on chicken meat from the design experiment.

Run	Factor		Survival of *S. aureus*/ Chicken Meat (Log_{10} CFU/ml)
	UV Treatment Time	US Treatment Time	
1	−1	−1	7.20 ± 0.03
2	+1	−1	6.70 ± 0.14
3	−1	+1	6.78 ± 0.36
4	+1	+1	0.00 ± 0.00
5	0	0	7.04 ± 0.33
6	0	0	7.08 ± 0.10
7	0	0	7.04 ± 0.08
Control 1	–	–	8.08 ± 0.07
8	−1	−1	7.15 ± 0.16
9	+1	−1	6.60 ± 0.44
10	−1	+1	6.78 ± 0.15
11	+1	+1	0.00 ± 0.00
12	0	0	7.00 ± 0.38
13	0	0	7.08 ± 0.18
14	0	0	7.08 ± 0.15
Control 2	–	–	8.14 ± 0.04

2.4. Microbiological analysis

This study used *S. aureus* for the inactivation experiments. Cultures were grown for 24 h at 37 °C in an incubator. The concentration of *S. aureus* cell cultures based on spectrophotometer readings at OD_{600}. OD_{600} of 1.0 = 8 × 108 cells/ml in PAW' dilution sample 10 ml for initial test conditions. For the counting of and *S. aureus*, serial dilutions of each sample solution (101) were plated on 3 M Petrifilm plates (3 M USA). Using Petrifilm Staph Express Count plates, the enumeration of *S. aureus* was carried out in accordance with AOAC Official Method 2003.11 (McMahon et al., 2003). (3 M USA). The Petrifilm plates were incubated at 35 °C for 24 h. All bacteria cell counts were performed in duplicate, and all results reported as a log reduction in mean values. Calculate the CFU value of the sample. Once count the.

colonies, multiply by the appropriate dilution factor to determine the number of CFU/ml in the original sample.

2.5. Verification of residual heavy metal in water

The analysis for copper (Cu) and iron (Fe) in your sample was performed by Central Laboratory (Thailand) Co., Ltd., located in Chiang Mai. They followed their internal method TE-CH-037, which is based on recognized water and wastewater testing standards set by APHA, AWWA, and WEF in the 22nd edition (2012) of their publication. Specifically, the methods used were likely 3030E, 3120B, and 3125B.

2.6. Color measurement

The study employed a Minolta CR-300 colorimeter to quantify the surface color changes in chicken and beef samples following treatment. The CIE Lab* system was utilized, where L^* represents lightness, a^* signifies redness/greenness, and b^* denotes yellowness/blueness. To assess the overall magnitude of color difference (ΔE*) induced by the treatment, the following equation was implemented (Han et al., 2014):

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

In this formula, ΔL^*, Δa^*, and Δb^* represent the absolute variations in the respective L^*, a^*, and b^* values between the pre-treatment and post-treatment samples.

2.7. Hardness measurement

Textural properties of chicken and beef samples were evaluated using a TA.XTplus texture analyzer. A flat-faced cylindrical probe (1.5 cm diameter) was employed to compress the samples to a strain of 70 % with a trigger force of 10 g. This approach quantified the hardness of the meat before and after treatment.

2.8. Analyze experimental results

Analysis of the best conditions for reducing food-borne pathogens by bringing the results of the ability of living cells to be analyzed by adopting ANOVA to help determine the impact on operating conditions.

2.9. Scanning electron microscopy (SEM) for S. aureus

Following centrifugation, the optimal PAW-treated and control samples were subjected to filtration through a sterile 25 mm polycarbonate filter with 0.2 μm track-etched pores (Whatman, GE Healthcare Life Sciences) mounted on a funnel assembly connected to a vacuum pump. The retained material was subsequently fixed and dehydrated. Imaging of the samples was performed using a SEM SU3800.

3. Results and discussion

This study investigated the combined effects of PAW and supplementary techniques on the physicochemical properties and pathogen survival rates. An experimental design was employed to evaluate the efficacy of MNB, UV and US in combination with PAW. Treatment time was chosen as the primary variable, with high and low levels established for each factor. The experimental design and results are presented in Table 2.

3.1. PAW combined with supplementary disinfection technique to the survival of S. aureus inactivation experiment

3.1.1. Investigation of the physicochemical properties and bactericidal activity of PAW in conjunction with a supplemental technique

Table 2 presents the surviving population of *S. aureus* after different plasma treatment conditions. The combination of PAW/MNB/UV and PAW/MNB/UV/US treatments resulted in the greatest reduction of *S. aureus*, achieving a 8.08 $\log_{10}$ CFU/ml decrease. Furthermore, the inhibition of pathogenic microorganisms was optimized when 10 min of MNB treatment time, 20 min of UV treatment time, and 20 min of US treatment time were employed.

3.1.2. Statistical evaluation of PAW combined with supplementary technique on survival of S. aureus

Table 3 shows the analysis of variance (ANOVA) of the factorial design experiment on PAW combined with a supplementary technique. The results indicate that only PAW combined with MNB, UV, and their two-way interactions (MNB*UV) significantly affected the survival rate of *S. aureus* ($p < 0.05$). The decision coefficient test (R-Square) of the response values from the ANOVA was used to determine the model's reliability. The coefficient of determination of the *S. aureus* quantification test result, R^2 and R^2 adjustment was 99.89 % and 99.85 %, respectively. Therefore, the model is more reliable and provides sufficient data to fit the equation and create a predictive model to identify the appropriate type that optimizes for reducing *S. aureus* infection (See Table 4).

A regression analysis is conducted to identify statistically significant factors influencing the process reaction. The coefficients associated with these factors are then incorporated into a predictive model. This model allows for the estimation of the process reaction based on future data generated by the program's analysis. Eq. 1 can be employed to identify the most appropriate value of treatment factors that can minimize the survival of *S. aureus*.

Survival of *S. aureus* = 6.1750–0.02625 UV-0.01975 US-0.013138 UV*US+1.6521 Ct Pt (1).

The factorial experiment allowed the development of a mathematical model (Eq. 1) to predict bacterial responses in future studies. This equation considers the influence of significant factors and their corresponding coefficients on the predicted response. For example, Eq. 1 predicts a *S. aureus* survival of 5.79 $\log_{10}$ CFU/ml under the lowest treatment conditions (0 min MNB, 0 min UV). Conversely, predicted survival drops to 0.00 $\log_{10}$ CFU/ml under the highest treatment conditions (10 min MNB, 20 min UV).

Table 3
ANOVA of *S. aureus* inactivation experiment based on factorial experimental design.

Source	DF	Adj SS	Adj MS	F-Value	*P*-Value
Model	5	61.3145	12.2629	9833.74	0.000
Blocks	1	0.0016	0.0016	1.29	0.289
Linear	2	38.1475	19.0738	15,295.43	0.000
UV	1	19.8765	19.8765	15,939.16	0.000
US	1	18.2710	18.2710	14,651.69	0.000
2-Way Interactions	1	13.8075	13.8075	11,072.37	0.000
UV*US	1	13.8075	13.8075	11,072.37	0.000
Curvature	1	9.3579	9.3579	7504.16	0.000
Error	8	0.0100	0.0012		
Lack-of-Fit	4	0.0046	0.0012	0.87	0.552
Pure Error	4	0.0053	0.0013		
Total	13	61.3245			

Table 4
Optimal values of *S. aureus* survival according to Response Optimization function.

Parameters						
Response	Goal	Lower	Target	Upper	Weight	Importance
Survival of *S. aureus*	Minimum		0	8.41	1	1

Solution				
Solution	UV	US	Survival of *S. aureus* Fit	Composite Desirability
1	20	20	−0.0000000	1

Contour plots (Figs. 1 and 2) were generated to visualize the relationship between the significant factors (MNB and UV treatment times) and the *S. aureus* survival response. These plots depict the optimal factor level combinations leading to the lowest bacterial survival. Darker blue regions in the contour plots indicate lower *S. aureus* populations, corresponding to plasma treatments with extended MNB and UV exposure (20 min each). Interestingly, the contour plots revealed no significant influence of US treatment time on *S. aureus* survival, as all US durations resulted in similar predicted survival levels (represented by a single line across the plots).

This investigation employed a response optimization technique to identify the experimental conditions that yielded the most favorable outcome. This statistical method pinpointed the key parameters that significantly impacted the response variable. The program required the user to specify the direction (minimize or maximize), optimal value, weight, and importance of each parameter. In this study, we aimed to minimize *S. aureus* concentration, setting a target value of 0 and an upper limit of 8.41 (control unit value). Both weight and importance were assigned a value of 1 (Table 4). The optimization analysis determined the most suitable settings for each factor to achieve minimal *S. aureus* infection. These settings were a UV treatment time of 20 min and no US treatment. This combination resulted in a minimum *S. aureus* value of 0.000 and a desirability function value of 1.0000. The high desirability score underscores the effectiveness of response surface methodology for identifying optimal conditions.

3.1.3. Experimental confirmation of optimal conditions for beef.

The analysis identified the most effective conditions to minimize *S. aureus* contamination. UV treatment for 20 min and US treatment for 20 min resulted in a complete elimination of *S. aureus* 0.000 $\log_{10}$ CFU/ml and achieved the optimal desirability of 1.000. This high desirability score emphasizes the significance of the response surface methodology in determining the ideal settings. Then apply the optimal conditions to beef. From the results of the experiment, it was found that under appropriate conditions, the *S. aureus* in beef could reach 8.20 $\log_{10}$ CFU/ml.

3.2. Nutritions experimemt

3.2.1. Verification of residual heavy metal in water

This study investigated the presence of heavy metals in process water (PAW) generated from a process. PAW was obtained from the Department of Industrial Engineering, Faculty of Engineering, Chiang Mai University, Thailand. The experiment specifically focused on comparing the concentrations of copper (Cu) and iron (Fe) in PAW with those found in tap water. The analysis revealed elevated levels of copper (Cu) and iron (Fe) in post-activation water (PAW) compared to pre-activation

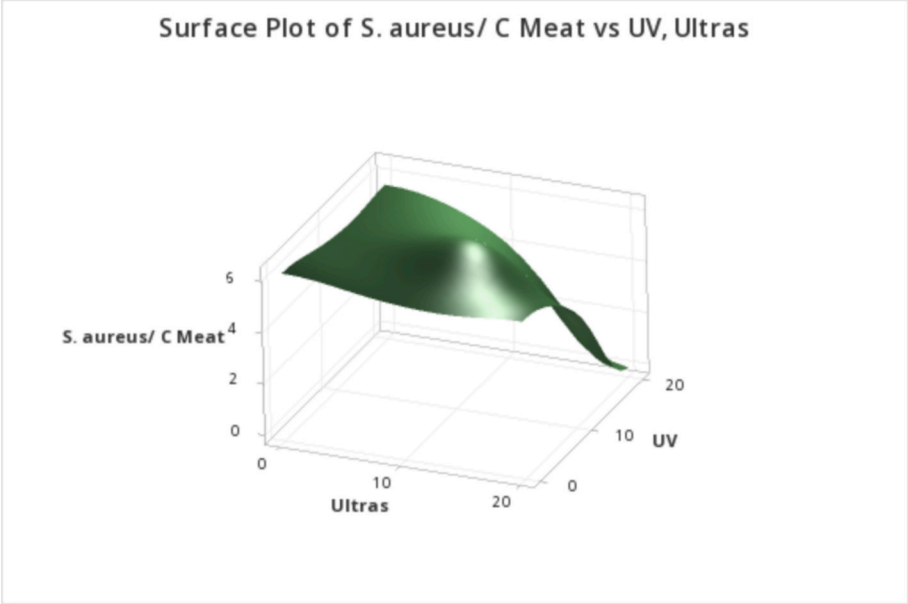

Fig. 1. Surface plot at MNB Treatment Time (A) and UV Treatment Time (B) to quantify *S. aureus*.

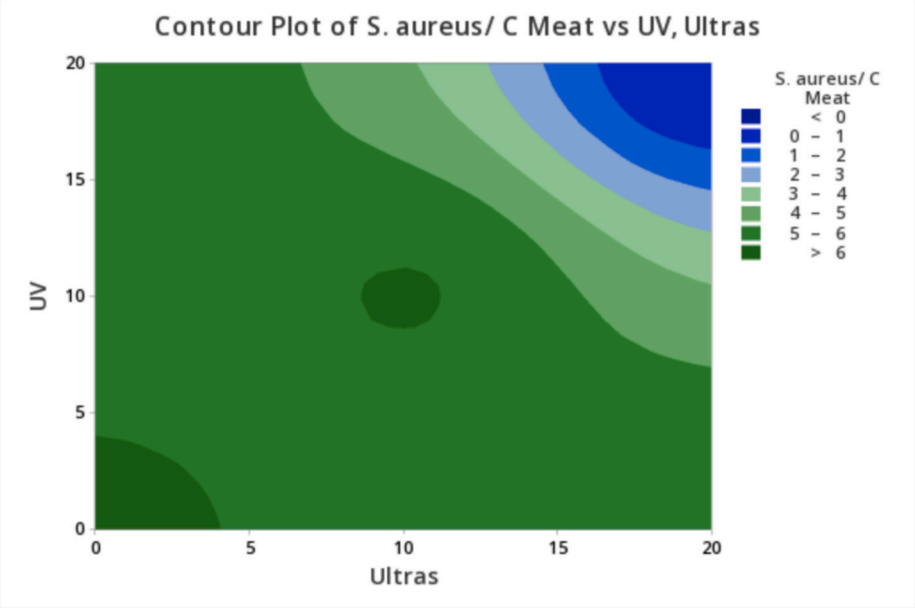

Fig. 2. Contour plot at MNB Treatment Time (A) and UV Treatment Time (B) to quantify *S. aureus*.

levels. These increases remained within the safety standards set by the Provincial Waterworks Authority (PWA), with Cu 0.080 mg/L not exceeding 2.0 mg/L and Fe 0.011 mg/L below 0.3 mg/L. The observed iron increase might support the occurrence of Fenton's reaction, potentially contributing to enhanced bacterial reduction efficiency. Nonetheless, the measured Cu and Fe concentrations remained well within the established safety limits, indicating the safety of PAW generated using supplementary techniques.

3.2.2. Color analysis

The average and standard deviation of L^*, a^*, b^*, and ΔE averages and standard deviations are displayed in Table 5. According to (Wrolstad & Smith, 2017), there are three categories for the perceptible color shift: "very distinct," "distinct," and "small differences." $\Delta E > 3$ corresponds to the most distinct color change. This study indicated that

Table 5
Color Analysis.

Treatment	L^*	a^*	b^*	ΔE
Optimal PAW' Condition in Chicken	55.87 ± 2.82	0.67 ± 0.57	9.91 ± 2.04	1.83 ± 2.33
Control Chicken	54.05 ± 0.83	1.67 ± 0.15	8.85 ± 0.70	
Optimal PAW' Condition in Beef	35.34 ± 1.05	13.14 ± 0.85	7.21 ± 0.64	1.50 ± 1.60
Control Beef	34.92 ± 1.12	12.45 ± 1.04	6.68 ± 1.15	

when chicken and beef was handled with PAW' condition, the ΔE values were "distinct." According to (Moreau et al., 2000), color changes should be clearly visible to the human eye when $1.5 < \Delta E < 3$. Yet, despite PAW's state, the sensory panel failed to detect any variations in the color of the chicken and beef.

3.2.3. Quality analysis of hardness measurement

The study investigated the effect of optimal PAW' condition on the hardness of chicken and beef meat. Chicken meat hardness did not show a significant difference between the optimal PAW' condition (85.97 ± 0.44 N) and the control condition (90.88 ± 0.23 N). Similarly, no significant difference in hardness was observed between the optimal PAW' condition (1221.67 ± 290.23 N) and the control condition (1268.33 ± 272.50 N) for beef. This outcome could have been caused by the ultrasonic bath's minimal energy dissipation into the system (Haughton et al., 2012). claim that while chicken flesh has a significant connective tissue content, the UV and US intensities were not strong enough to modify the characteristics of the meat and may have even been too low to affect the texture.

3.3. Plasma-induced S. aureus inactivation from SEM images

SEM analysis revealed visible damage to the surface of *S. aureus* cells treated with PAW and supplementary techniques, indicative of cell disruption(Fig. 3a). This contrasts with the intact, normal morphology observed in the control group (Fig. 3b). These findings suggest that PAW, in combination with supplementary treatments, induces irregular cell shape and shrinkage in *S. aureus*.

4. Discussion

The research aimed to assess the impact of plasma on enhancing efficiency by exploring and assessing PAW MNB UV and US technologies. A PAW generation experiment was conducted as part of a two-part investigation that included other disinfection methods (MNB UV and US technology). This study employed a representative number of prevalent foodborne pathogens throughout the experimentation. Following the initial experiment's optimization, treated water designated as PAW' produced under these optimal conditions for the subsequent experiment. This second experiment assessed the efficacy of PAW' against chicken meat contaminated with a foodborne illness during processing. Furthermore, PAW' combined with UV and US disinfection techniques in the second experiment to explore the synergistic effects of these additional approaches on the inactivation of pathogenic bacteria, exemplified by *S. aureus*. Notably, the first experiment MNB was not carried over to PAW'. This exclusion is due to the potential for MNB persistence within the treated water throughout the extended processing duration. The research looked at pathogen decrease. Researchers are using PAW in conjunction with a supplementary disinfection technique consisted of three variables: MNB Treatment Time, UV Treatment Time (min), and US Treatment Time (min) in a factorial experimental design.

The experimental findings demonstrate a statistically significant reduction in *S. aureus* levels by 8.41 $\log_{10}$ CFU/ml across both PAW/MNB/UV and PAW/MNB/UV/US treatment groups. Notably, the greatest suppression of microbial pathogens was achieved when both UV and US exposure times were extended to 20 min. RONS are the main inactivation agents in non-thermal plasma. This research investigates the role of ROS, particularly long-lived species like H_2O_2, in PAW and their contribution to bacterial inactivation. A four-step mechanism is proposed to explain the bactericidal effect. The first step involves the underwater plasma generation of significant amounts of ROS. Subsequently, these ROS species, particularly H_2O_2, target the bacterial cell membrane's lipid bilayer in step two. This oxidation disrupts cell permeability and leads to membrane depolarization. In the third stage, ROS pass through temporary pores and cause oxidative stress in the cell, causing intracellular ROS to rise. During stage four, intracellular ROS were observed to interact with cellular biomolecules, including proteins, lipids, and carbohydrates. This interaction resulted in structural alterations and modifications to chemical bonds within these molecules. Interestingly, the study also found that the addition of organic matter to the PAW solution demonstrably reduced ROS levels. The combined effect of elevated intracellular ROS concentrations and a decreased cellular pH was hypothesized to induce redox reactions within the cell. These redox reactions were further postulated to disrupt cellular pH homeostasis, ultimately leading to cell death. (Zhang, Ma, et al., 2016).

PAW results from non-thermal atmospheric plasma (NTAP) reacting with water and contains a wide range of highly ROS and RNS (Perez et al., 2019; Zhou et al., 2019). In food decontamination, both ROS and RNS are essential factors. Reactive Oxygen Species (ROS) play a significant role as signal molecules in different biological cells. It knows that ROS can boost inner oxidative stress and programmable cell death. Synergistic antimicrobial activity of PAW (Maher et al., 2020). Fresh chicken flesh is cleaned and disinfected using PAW, a plasma technology application (Royintarat et al., 2019). The textural property of hardness

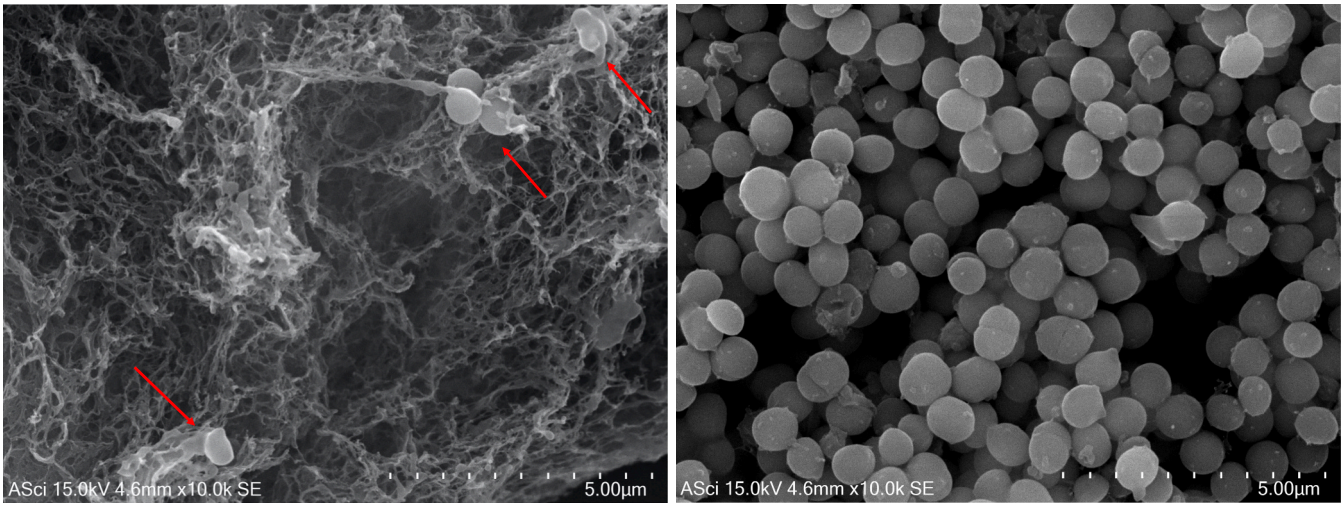

Fig. 3. SEM images of PAW with supplementary techniques and control and treated *S. aureus* cells after exposure with PAW with supplementary treatment (Optimal condition) and Tap water (Control) for 1 h following 24 h of posttreatment storage. (a) Treated *S. aureus* with PAW and supplementary treatment; (b) *S. aureus* with Tap water.

in beef is primarily dictated by the intactness of its protein structure. However, continuous degradation of these proteins by endogenous and exogenous microbial enzymes leads to a decline in hardness throughout storage (Lee JuRi et al., 2018). As a result, more study is needed to increase the efficacy of disinfection in chickens. In the investigation, MNB technology with high effectiveness reactive species transfer from PAW was chosen.

PAW/MNB and PAW/MNB/US treatments achieved the greatest reduction in *S. aureus*, by 0.35 $\log_{10}$ CFU/ml. In this case, MNB was consequently combined with low pH PAW. *S. aureus* measurement data was analyzed in the context of the previously identified significant factor. The results of an *S. aureus* inactivation test were used to create a survival of *S. aureus* between PAW combined with MNB in plasma treatment. This result was consistent with the previous study (Moonsub et al., 2024), when combined PAW with MNB, this approach facilitates enhanced mass transfer of biochemically reactive species generated by the plasma to the targeted microbial cells within the solution, as well as the biological activity and selectivity of the resulting activated solutions against pathogenresiding microorganisms (Baek et al., 2020; Govaert et al., 2019; Moonsub et al., 2022; Zhou et al., 2019). MNB, characterized by their high specific interfacial area, extended residence time, and elevated internal pressure, facilitate enhanced mass transfer between the gas and liquid phases. This property suggests their potential application in encapsulating species produced by the cold atmospheric pressure plasma (CAPP) process, potentially leading to improved efficiency within existing reactors. Additionally, the exposure of these planktonic bacterial cells to PAW, known for its abundance of ROS and RNS, serves as a relevant context for further investigation. These species may interact with microbial membrane proteins, DNA, and metabolic enzymes, disrupting vital cellular functions and structures and producing potent antimicrobial efficacy.

The presence of RONS influences the physicochemical characteristics PAW containing MNB. The steps that culminate in the creation of H_2O_2 (Feizollahi & Roopesh, 2021). The observed increase in ORP can be attributed to the application of PAW. This is likely due to the presence of a higher concentration of active ions and oxidizing species within PAW compared to the MNB. (Rathore & Nema, 2021). It should be noted that pH has an effect on the ORP; at acidic pH, hydrogen ions (H^+) produce an increase in the ORP. We measured the H_2O_2 content in order to determine the physicochemical properties of PAW that had been kept for 2 h. The principal causes of H_2O_2 production were water breakdown and subsequent •OH radical recombination, the H_2O_2content reduced during storage (Rathore & Nema, 2021).

In summary, we established empirically and theoretically that Henry's law coefficient is not the only factor affecting the dissolution of highly and weakly soluble (H_2O_2 and O_3) (Hassan et al., 2021). RONS are the main inactivation agents in non-thermal plasma. Zhang et al., (2016) focuses on ROS in PAW, exceptionally long-lived ROS such as H_2O_2, which are responsible for bacterial survival. Low pH and excessive intracellular ROS triggered redox reactions within the cell, upsetting pH homeostasis and leading to cell death (Zhang et al., 2006). The primary inactivation agents are ROS. ROS from the PAW production process is substantial. As a consequence, UV technology was selected to increase reactive species.

The PAW/US treatments decreased *S. aureus* by 0.22 $\log_{10}$ (CFU/ml). As a result, the optimal condition *S. aureus* survival population in chicken and beef at 0.00 and 0.00 $\log_{10}$ CFU/ml, respectively When compared to the chicken and beef control at 8.41 and 8.20 $\log_{10}$ CFU/ml, respectively were discovered when PAW treatment was combined with UV and US for 20 and 20 min of treatment. A survival of *S. aureus* between the PAW combined with US in plasma treatment and an *S. aureus* inactivation test was created using the result. This result was consistent with the previous study, The US implemented modifications to the samples, resulting in an enhanced PAW absorption rate. The germs on the chicken muscle displayed severe physical damage compared to the bacteria on the skin because the skin had more organic material than the muscle did. The generation of ROS and RNS can be inhibited by a high concentration of omega-3 and omega-6 PUFAs. The ROS are interested in C—H bonds, mainly double bonds, because abstracting a hydrogen atom requires less energy (272 kJ/mol) than rehashing another C—H bond (422 kJ/mol). Consequently, the antibacterial efficacy of ROS against bacteria on chicken skin diminished. The PAW with supplementary technology employed in this study demonstrated superior efficacy in eradicating *S. aureus* bacteria compared to the cold plasma approach utilized by (Mai-Prochnow et al., 2016). Our findings indicate a reduction in pathogens by 8.41 $\log_{10}$ CFU/ml, surpassing the antibacterial capabilities of the aforementioned research. In contrast, the previous study achieved reductions of only 0.6 $\log_{10}$ CFU/ml for *Bacillus subtilis*, 1 log10 CFU/ml for *Staphylococcus epidermidis*, and 2 log10 CFU/ml for *Kocuria carniphila*. The results of our investigation demonstrate that the novel system is more effective in eradicating gram-positive pathogens than previously reported methods. This observation is evidenced by the protrusion of the bacterial membrane, suggesting a stress response, rather than complete bacterial disintegration, which would be indicative of cell death on chicken and beef muscle. The US process disrupted the membrane, releasing unsaturated lipids and interacting with free radicals in PAW. Lipid oxidation is the method most commonly used. Most lipid oxidation reactions occur in muscle foods, resulting in color, odor, taste, and shelf-life changes. Fresh pork and beef treated with dielectric barrier discharge plasma, pork loin treated with helium and oxygen plasma, and ground pork treated with plasma jet are only a few investigations that have discovered lipid oxidation (Royintarat et al., 2020). The integrity and potential of microbial cell membranes could also be disrupted by PAW, leading to the release of internal substances such proteins and nucleic acids (Reece et al., 2017; Zhang et al., 2013).

US-assisted PAW for bacterial inactivation in Poultry Industry has also been studied. Instead of using chlorine solution for bacteria inactivation during the slaughtering process, PAW combined with US for both *E.coli* and *S.aures* inactivation has been implementedv (Royintarat et al., 2019). The PAW system in combination with supplemental approach is shown to have physicochemical characterization and bactericidal effects in the investigation. The outcome reveals that the PAW with UV, with a value of 240 mS/cm, has the highest concentration of EC. The PAW with MNB produces the greatest ORP, which is 361.92 mV, according to ORP measurements. Last but not least, the H_2O_2 concentration test shows that PAW treatment using all combination procedures results in the highest H_2O_2 concentration. In line with prior research, this study employed PAW, a promising technology within the field of plasma medicine, to achieve bacterial inactivation on fresh raw chicken meat. PAW offers a distinct advantage due to its chemical-free nature, making it a versatile tool applicable in various sectors including medicine, agriculture, and food processing. The investigation combined PAW generated via arc liquid discharge with US to assess its efficacy against *S. aureus*. The findings demonstrated a significant reduction in *S. aureus* (1.51 $\log_{10}$ CFU/ml) on 4 mm thick chicken muscle samples when the combined treatment was employed. Notably, US treatment alone resulted in a considerably lower reduction (0.85 $\log_{10}$ CFU/ml), highlighting the synergistic effect of the combined approach. Furthermore, PAW alone exhibited a moderate reduction in *S. aureus* (0.74 $\log_{10}$ CFU/ml), suggesting its independent antimicrobial potential. (Royintarat et al., 2020) which PAW increase efficiency up to 8.41 $\log_{10}$ CFU/ml of *S. aureus* shown in Table 6.

This result was consistent with the previous reports, PAW-US appropriate preservation procedures are thus required to maintain microbiological safety and fresh produce quality. For the preservation of fruits and vegetables, many new processing technologies (such as high hydrostatic pressure, cold plasma, US, and UV radiation) have been utilized in recent years. It has been proven that combining PAW with other technologies (e.g., moderate heat and US) enhances antibacterial effectiveness synergistically (Royintarat et al., 2020). The sequential combination of washing with PAW and moderate heating at 60 °C. The

Table 6
Comparison between current technology and others technology.

List of technology	Efficiency	Bacterial Reduction	Researcher
PAW with MNB UV and US	The new integrated system was able to reduce *S. aureus*, which is increase effective in inhibition bacteria than using normal plasma water.	8.41 $\log_{10}$ CFU/ml of *S. aureus*	Current study
Cold Atmospheric Plasma (CAP)	Cold atmospheric plasma (CAP) reduction for *S. aureus* counts.	0.85 $\log_{10}$ CFU/ml of *S. aureus*	(Gök et al., 2019)
Dielectric Barrier Discharge Atmospheric Cold Plasma (DBD-ACP)	The inactivation level was inversely proportional to the Dielectric Barrier Discharge Atmospheric Cold Plasma (DBD-ACP) treatment.	1.07 $\log_{10}$ CFU/ml of *S. aureus*	(Liao et al., 2017)
High-Voltage Atmospheric Cold Plasma (HVACP)	High-Voltage Atmospheric Cold Plasma (HVACP) inactivation was declined *S. aureus*.	1.8 $\log_{10}$ CFU/ml of *S. aureus*	(Han et al., 2016)
PAW with US	PAW(normal) in decreased *S. aureus*.	0.74 $\log_{10}$ CFU/ml of *S. aureus*	(Royintarat et al., 2020)
PAW generated	PAW treatment produced reduction of pathogenic species (*E. coli*, *Salmonella enterica*, *L. monocytogenes*) on the cucamelon surface.	3 $\log_{10}$ CFU/g of pathogenic species (*E. coli*, *Salmonella enterica*, *L. monocytogenes*)	(Rothwell et al., 2023)
Cold Atmospheric Plasma (CAP)	Atmospheric cold plasma (ACP) treatment demonstrated a significant reduction in *L. monocytogenes* and *V. parahaemolyticus* populations on both fresh and frozen shrimp.	0.94 and 1.26 $\log_{10}$ CFU/g of L. *monocytogenes* 1.68 and 2.0 log10 CFU/g for *V. parahaemolyticus* (fresh and frozen shrimp)	(Liu et al., 2024)
Plasma-Treated Liquid (PTL)	Plasma discharge exhibited significant bactericidal activity against *S. aureus*, reduction in bacterial viability following a plasma treatment.	3 $\log_{10}$ CFU/ml of *S. aureus*	(Lavrikova et al., 2024)

background microbiota populations injected food-borne pathogens on shredded slanted Chinese cabbages dramatically decreased (Kaushik et al., 2019). The quality of salted Chinese cabbage was not affected by this combination treatment. In recent research, PAW, in combination with US, was shown to be more successful than either approach alone at reducing *S. aureus* on chicken flesh and skin (Royintarat et al., 2019). The antibacterial activity and membrane damage mechanism of PAW against *Pseudomonas deceptionensis*. Various innovative non-thermal processing processes have been developed and employed in the food business to fulfill the rising customer demand for healthier, safer, and fresher meals (Xiang et al., 2018). Non-thermal processing methods include high hydrostatic pressure, electrolyzed water, pulsed light, US technology, pulsed electric fields, dense phase carbon dioxide, ionizing radiation, and cold plasma. The shelf life of processed foods while preserving their nutritional value and sensory qualities. Mechanism and applications for fresh produce sanitation. Fruits and vegetables are necessary for optimum health because they are rich sources of critical vitamins, minerals, and dietary fiber (Reece et al., 2017).

5. Conclusions

A factorial design was employed to optimize the experimental conditions for microbial disinfection. Initial findings, based solely on *S. aureus* inactivation, PAW alone exhibited the most promising results in eliminating all bacterial specimens. Nonetheless, when *S. aureus* was tested on chicken, the results showed that PAW with MNB UV and US had the most effective. PAW with MNB at 10 min and UV at 20 min was the optimal condition to inactivate of *S. aureus* in chicken and beef up to 8.41 and 8.20 $\log_{10}$ CFU/ml, respectively. For the only microbial and chicken meat experiment, PAW and UV and US was essential factor affecting the bacteria survival ($p < 0.05$). The optimal condition of process to inactivate PAW with UV of 20 min and US 20 min up to 8.41 and 8.20 $\log_{10}$ CFU/ml of *S. aureus* in chicken and beef. In addition, combining PAW and combination techniques could improve the performance of bacteria inactivation. Further investigation will focus on the impact assessment of the combination technique of PAW and the combination of a variety of meat. PAW with supplementary techniques can generated ROS in system.

CRediT authorship contribution statement

Kochakon Moonsub: Writing – review & editing, Writing – original draft, Validation, Formal analysis, Data curation, Conceptualization. **Phisit Seesuriyachan:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Dheerawan Boonyawan:** Supervision, Conceptualization. **Pornchai Rachtanapun:** Supervision. **Chonchar-oen Sawangrat:** Supervision, Funding acquisition. **Takron Opassu-wan:** Supervision. **Wassanai Wattanutchariya:** Writing – review & editing, Validation, Investigation, Conceptualization.

Declaration of competing interest

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

Data availability

No data was used for the research described in the article.

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