SYNERGISTIC ENHANCEMENT OF PHYSICOCHEMICAL PROPERTIES BY COMBINING PLASMA-ACTIVATED WATER, MICRO/NANOBUBBLES, AND CHEMICAL SOLUTIONS

Yosita Phanluck[1,2], Phisit Seesuriyachan[1,3], Dheerawan Boonyawan[4], and Wassanai Wattanutchariya[1,*]

Received: December 26, 2024; Revised: February 24, 2025; Accepted: April 21, 2025

Abstract

Plasma-activated water (PAW) shows promise in agriculture, food production, and medicine by inducing physicochemical changes through reactive species during plasma treatment. This study aimed to enhance PAW efficiency by combining a chemical solution with PAW and micro/nanobubbles (MNBs), optimizing the ratio of chlorine (Cl), sodium chloride (NaCl), and lactic acid (LA) using mixture design. PAW underwent 20 min of treatment, while MNB treatment lasted 10 min. An examination of physicochemical properties, including pH, oxidation-reduction potential (ORP), electrical conductivity (EC), and hydrogen peroxide (H_2O_2) concentration revealed a significant 10- to 20-fold increase in H_2O_2 concentration compared to the control without chemicals. The combined treatment created an acidic environment (pH ranging from 3.59 to 4.36), an ORP range of 395 to 456 mV, and high EC values between 360 to 1,917 µS/cm. The optimal chemical solution ratio was Cl (17.80%), NaCl (28.28%), and LA (53.92%). Model predictions indicated a maximum H_2O_2 concentration of 152.98 mg/L, while actual data showed 171 mg/L – a 12% increase – with no statistically significant difference from the model prediction. These findings underscore the effectiveness of combining chemical solutions with PAW and MNBs for microbial control, offering an innovative approach for the food industry to enhance antimicrobial action, extend product shelf-life, and ultimately improve food safety.

Keywords: **Chemical Solution; Hydrogen Peroxide; Micro/Nanobubbles; Mixture Design; Physicochemical Properties; Plasma-Activated Water**

---

[1] *Advanced Technology and Innovation Management for Creative Economy Research Group, Department of Industrial Engineering, Faculty of Engineering, Chiang Mai University, Chiang Mai, Thailand. E-mail: wassanai@eng.cmu.ac.th; yosita.phanluck@gmail.com*
[2] *Department of Industrial Engineering, Faculty of Engineering and Technology, North-Chiang Mai University, Chiang Mai, Thailand. E-mail: yosita.phanluck@gmail.com*
[3] *Division of Biotechnology, Faculty of Agro-Industry, Chiang Mai University, Chiang Mai, Thailand. E-mail: phisit.s@cmu.ac.th*
[4] *Plasma and Beam Physics Research Facility, Chiang Mai University., Chiang Mai, Thailand. E-mail: dheerawan.b@cmu.ac.th*
[*] *Corresponding author*

DOI: https://doi.org/10.55766/sujst8053

Introduction

Historically, methods for removing contaminants such as dirt, chemicals, and microorganisms from product surfaces primarily involved washing with clean or tap water. However, these methods often failed to completely eliminate contaminants, particularly resilient microorganisms capable of surviving water-based cleaning, leading to their persistence on the product. Even small amounts of contaminated products can pose health risks when ingested, potentially causing symptoms such as nausea, vomiting, headaches, and diarrhea. In cases of severe contamination, ingestion may result in breathing difficulties, muscle spasms, fatigue, and loss of consciousness, increasing the risk of severe illness or even death. Additionally, contaminants accelerate product deterioration, reducing shelf life and leading to spoilage. Implementing a more effective method for contaminant removal could improve consumer health and extend product longevity.

In recent years, there has been a significant increase in the development and application of various methods for eradicating microorganisms. These methods utilize a range of technologies, including chemical methods (Francis and O'Beirne, 2005; Velázquez *et al*., 2009; Ramos *et al*., 2020), electrolyzed water (Selma *et al*., 2008; Graça *et al*., 2011), UV technology (Ben-Yehoshua and Mercier, 2005), and one of the most advanced technologies - plasma technology.

Plasma is one of the four fundamental states of matter, along with solid, liquid, and gas. It is a highly ionized and electrically conductive gas composed of positively charged ions and free electrons. In a plasma state, some or all of the electrons have been stripped from their parent atoms or molecules, creating a mixture of charged particles. This ionization occurs due to high temperatures or the application of strong electric fields. Plasma can conduct electricity and generate reactive oxygen species (ROS) and reactive nitrogen species (RNS), collectively known as reactive oxygen and nitrogen species (RONS). Some examples of RONS include hydroxyl radicals ($\cdot OH$), hydrogen peroxide (H_2O_2), ozone (O_3), superoxide ($\cdot O_2^-$), nitric oxide ($NO\cdot$), and peroxynitrite (ONOOH), all of which exhibit antimicrobial properties. The generation of reactive species in plasma can generally be described using Equations 1-9 from Pârvulescu *et al*. (2012). Because of these properties, plasma technology is widely applied in microbial inactivation (Hänsch *et al*., 2015; Xiang *et al*., 2019; Bai *et al*., 2020; Qian *et al*., 2020; Royintarat *et al*., 2020; Rothwell *et al*., 2022), surface treatments (Oh *et al*., 2011; Mai-Prochnow *et al*., 2021; Choi *et al*., 2022), food processing (Ma *et al*., 2015; Sruthi *et al*., 2022; Jyung *et al*., 2023), agriculture (Thirumdas *et al*., 2018; Darmanin *et al*., 2020; Fan *et al*., 2021), and other fields.

$$O_2 + e^- \rightarrow 2O + e^- \quad (1)$$
$$O_2 + O \rightarrow O_3 \quad (2)$$
$$N_2 + e^- \rightarrow 2N + e^- \quad (3)$$
$$N + O \rightarrow NO \quad (4)$$
$$NO + O \rightarrow NO_2 \quad (5)$$
$$O_3 + NO \rightarrow NO_2 + O_2 \quad (6)$$
$$H_2O + e \rightarrow H^+ + \cdot OH + 2e^- \quad (7)$$
$$\cdot OH + \cdot OH \rightarrow H_2O_2 \quad (8)$$
$$H_2O_2 + H^+ + NO_2^- \rightarrow ONOOH + H_2O \quad (9)$$

Plasma-activated water (PAW) is water that has been exposed to a plasma discharge, leading to the generation of ROS. These highly reactive species damage and destroy the cell walls of microorganisms, leading to cell death. PAW holds potential for applications in disinfection, water treatment, and other processes that benefit from the presence of reactive species. Additionally, PAW is being explored for its ability to eliminate contaminants, microorganisms, and other unwanted substances, making it a promising technology for healthcare, agriculture, and environmental remediation.

Micro/nanobubbles (MNBs) technology is a high-performance oxidation method recognized for its ability to remove non-biodegradable contaminants. Microbubbles typically range in size from 10 to 50 μm in diameter, while nanobubbles are smaller than 200 nm (Agarwal *et al*., 2011). These tiny bubbles possess unique properties compared to conventional larger bubbles, including high stability, slow dissolution, and a large surface area that facilitates the rapid transfer of reactive species. Due to their small size, MNBs can move quickly and disrupt cell walls (Hu and Xia, 2018). Moreover, they remain in water for extended periods, prolonging microorganism exposure to reactive species (Wang *et al*., 2020).

Combining PAW with MNBs increases the concentration of H_2O_2 among the ROS, enhancing its antimicrobial potential. PAW generates H_2O_2, which naturally possesses antimicrobial properties, and MNBs amplify its impact by aerating and oxygenating the surrounding water, increasing the surface area available for chemical reactions, resulting in a faster, more efficient process (Moonsub *et al*., 2022). Additionally, MNBs encapsulate H_2O_2 produced by PAW, allowing for more controlled chemical reactions that improve its physicochemical properties by creating a more stable H_2O_2 system. As a result, H_2O_2 remains in the water longer and moves more easily across cell membranes (Moonsub *et al*., 2024), allowing deeper penetration into microbial cells and effectively disrupting their structures and causing eventual cell death. Previous studies have demonstrated that combining PAW with MNBs effectively increases H_2O_2 concentration and enhances its ability to reduce *Escherichia coli* levels. Specifically, H_2O_2 concentration increased to 13.54 mg/L, reducing *E. coli* from 6 log_{10} CFU/mL to 5.65 log_{10} CFU/mL (Moonsub *et al*., 2022; 2024). However, despite the improved H_2O_2 concentration achieved through this combination, its antimicrobial potential remained insufficient to fully neutralize all microorganisms. Therefore, the addition of chemicals was considered necessary to further enhance H_2O_2 concentrations and improve antimicrobial effectiveness.

Chlorine (Cl), sodium chloride (NaCl), and lactic acid (LA) are widely used as antimicrobial agents in chemical solutions due to their long-standing application in the food industry, as well as their relatively low cost and easy availability. However, some of these chemicals may leave residues that are harmful to consumers. We propose an antimicrobial solution consisting of Cl, NaCl, and LA treated with PAW and MNBs to enhance both efficiency and safety in food industry applications. This approach focuses on the generation of H_2O_2 and the use of MNBs to increase surface area and produce synergistic effects, distinguishing it from previous studies that have employed these solutions using traditional methods. For example, washing contaminated lettuce with a Cl solution can reduce bacterial contamination by 1.6 log_{10} CFU/g, which is considered a low antimicrobial efficiency and may leave harmful residues (Ramos *et al*., 2020). In contrast, our proposed method offers higher antimicrobial efficiency due to the effects of H_2O_2, while also preventing the formation of harmful residue on food.

H_2O_2 is an antimicrobial agent used across various industries. In industrial processes, H_2O_2 is commonly used for bleaching, cleaning, and disinfecting water systems. Currently, its use in the food industry remains limited due to concerns about residual contamination. Therefore, we propose producing H_2O_2 from chemical solutions combined with PAW and MNBs for use in the food industry. This method is safer, as PAW is an environmentally friendly technology. The resulting H_2O_2 naturally decomposes back into water, leaving no harmful residues in food. Additionally, MNB technology facilitates H_2O_2 transfer, making food disinfection faster and more efficient; it also increases the concentration of H_2O_2 by utilizing safe chemical solutions within the levels specified by regulatory agencies. Thus, H_2O_2 produced from chemical solutions combined with PAW and MNBs offers significant benefits to the food industry by addressing food safety challenges.

Currently, no research has been published on chemical solutions treated as PAW combined with MNBs. This research focuses on studying the synergistic effects of Cl, NaCl, and LA treated with PAW and MNBs to enhance physicochemical properties and determining the optimal chemical solution ratio using mixture design with the Minitab program. The physicochemical properties examined include pH, oxidation-reduction potential (ORP), electrical conductivity (EC), and H_2O_2 concentration, all of which play significant roles in the antimicrobial properties of chemical solutions with PAW and MNBs. This approach presents the advantages of our H_2O_2 production method to the food industry by improving antimicrobial efficacy and extending product shelf life.

Materials and Methods

Sample Solutions Preparation

Calcium hypochlorite at a concentration of 200 ppm (65% w/v, World Chemical, Thailand) was used as a source of Cl for this experiment. NaCl at a concentration of 0.85% w/v (AR Grade, Ajax Finechem, Australia) and LA at a concentration of 0.2% w/v (85% w/v; AR Grade, Ajax Finechem, Australia) were also used. These solutions were prepared according to a model mixture design ratio with a total volume of 200 ml, and the pH was adjusted to neutral.

Experimental Design

The experimental design, which investigates the effects of chemical solutions on physicochemical properties, is shown in Table 1.

Table 1. The ratio of chemical solutions

Cl (%)	NaCl (%)	LA (%)	Condition
100	0	0	Cl
0	100	0	NaCl
0	0	100	LA
50	50	0	Cl*NaCl
50	0	50	Cl*LA
0	50	50	NaCl*LA
33.33	33.33	33.33	Cl*NaCl*LA
66.67	16.67	16.67	Cl*CL*NaCl*LA
16.67	66.67	16.67	Cl*NaCl*NaCl*LA
16.67	16.67	66.67	Cl*NaCl*LA*LA

The symbol * indicates interaction between variables

The experimental setup includes three replicates, totaling 30 experiments, conducted at a 95% confidence level using Minitab 18 statistical software (Minitab, LLC in PA, USA).

PAW Generation Combined with the MNB System

This experiment utilized a PAW system combined with an MNB system, with MNBs being applied before PAW, as shown in Figure 1. This approach builds on previous research, demonstrating that using MNBs before PAW resulted in a maximum H_2O_2 concentration of 6.13 mg/L (Moonsub *et al*., 2022). The fabrication process began with the setup of an in-house manufactured MNB system, as shown in Figure 2(c). Micro/nanobubbles were generated by drawing 200 mL of the sample solution and surrounding ir into the apparatus. The solution was circulated using a centrifugal pump at a pressure of 3 bar for 10 min, maintained at 25°C, producing a high volume of micro/nanobubbles. Following MNB

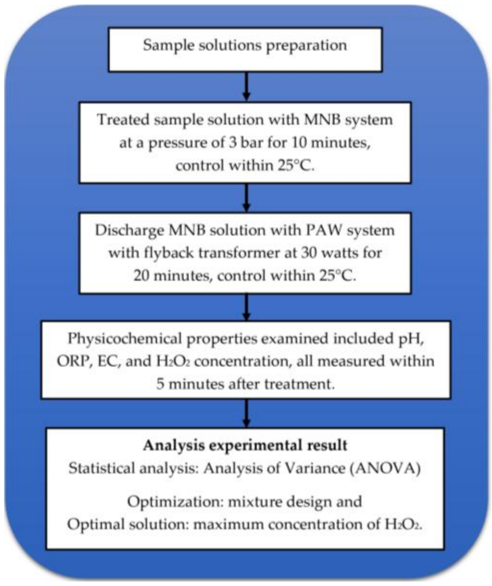


Figure 1. Scheme for PAW generation combined with the MNB system

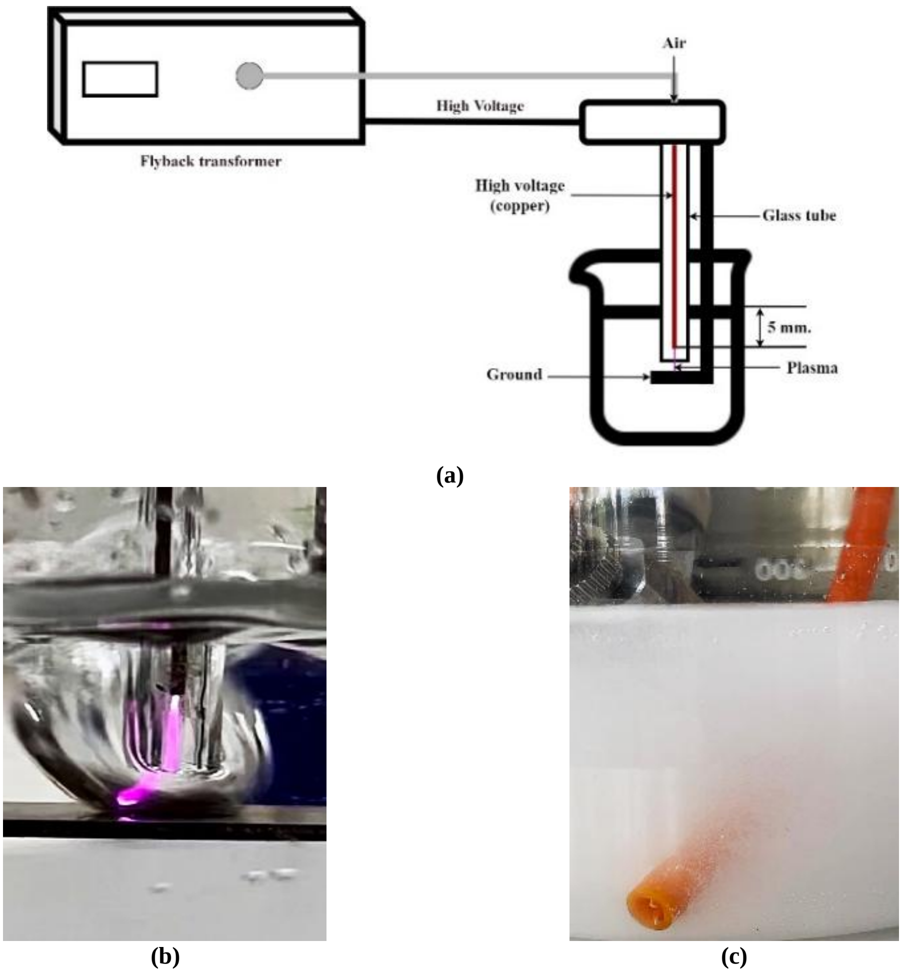
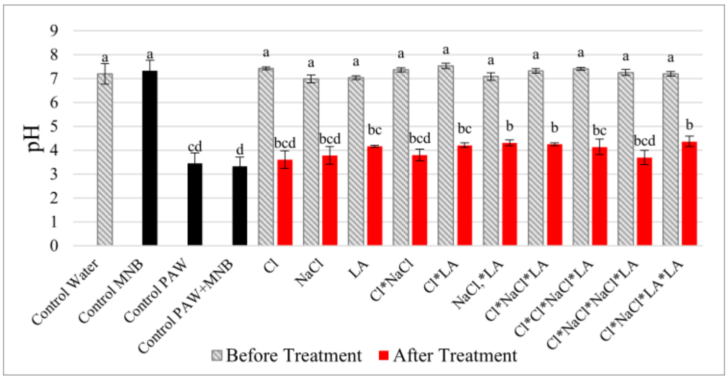


Figure 2. (a) Schematic of the PAW setup, (b) PAW generation, and (c) MNB generation

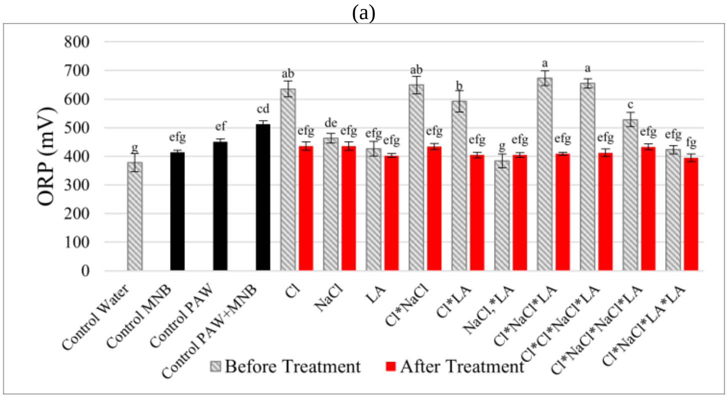
treatment, the PAW system was then set up. This system employed a plasma jet connected to a flyback transformer to generate PAW, a method successfully used in previous research (Royintarat *et al*., 2019b; Moonsub *et al*., 2022), as shown in Figures 2(a) and 2(b). The device consisted of a copper wire as the single electrode, a glass tube, and a stainless-steel ground. PAW was generated by powering the plasma jet with a 30-watt flyback transformer and supplying air into the 200 mL sample solution for 20 min, maintained at 25°C. Plasma discharge occurred just below the water surface, with a 5 mm distance between the electrode tip and the solution surface, producing the maximum H_2O_2 concentration.

Physicochemical Properties

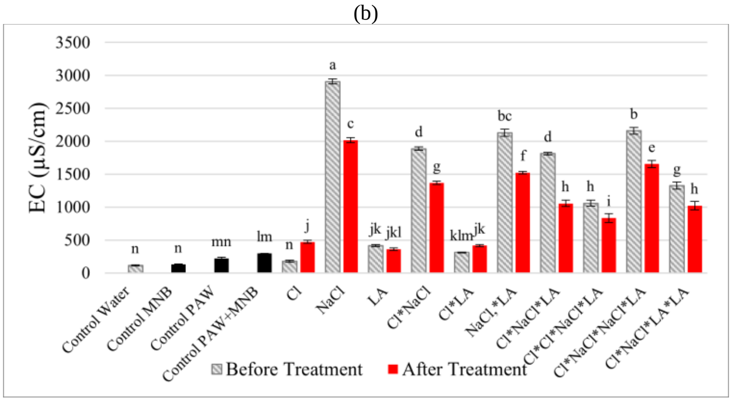
The pH, ORP, EC, and H_2O_2 were measured within 5 min after PAW and MNBs. The pH, ORP, and EC values were measured using a pH/ORP



(a)



(b)



(c)

Figure 3. The physicochemical properties: (a) pH, (b) ORP, and (c) EC. Error bars indicate standard deviation (n = 3). Small letters: Same letters indicate no significant difference among treatments (*P*>0.05), while different letters indicate significant difference among treatments (*P*<0.05). Abbreviations: PAW = plasma-activated water; MNB = micro/nanobubbles; Cl = Chlorine; NaCl = Sodium Chloride; LA = Lactic Acid

tester (LAQUA PH1100, Horiba, Japan) and an EC tester (LAQUAtwin-EC-11, Horiba, Japan), respectively. The H_2O_2 concentration was measured using a hydrogen peroxide test. The treated sample solution was mixed with the test reagent and allowed to stand for 10 min, after which the light intensity was measured using the photometric method (Spectroquant®, Merck KGaA, Darmstadt, Germany).

Statistical Analysis

The experiment was conducted using a mixture design. All analyses were performed in triplicate, and the results were presented as mean values ± standard deviation (SD). H_2O_2 concentration was selected as a representative of antimicrobial potential in this experiment. Analysis of variance (ANOVA) and response optimization analysis were used to evaluate the impact of each factor and interaction at $P \leq 0.05$, utilizing Minitab 18.

Results and Discussion

To examine the synergistic effects of chemical solutions with PAW and MNBs, as well as the control without chemicals, an experimental design based on the ratio of chemical solutions was employed. The physicochemical properties were analyzed by measuring pH, ORP, EC, and H_2O_2 concentration. The results are as follows:

Effect of Chemical Solutions with PAW and MNBs on pH, ORP, and EC

Figure 3(a-c) presents the physicochemical properties of the chemical solutions before and after treatment with PAW and MNBs, as well as the control without chemicals. The experimental results indicate that after treatment, the pH values of the solutions ranged from 3.59 to 4.36. Additionally, ORP values ranged from 395 to 456 mV, while EC values ranged between 360 to 1,917 µS/cm.

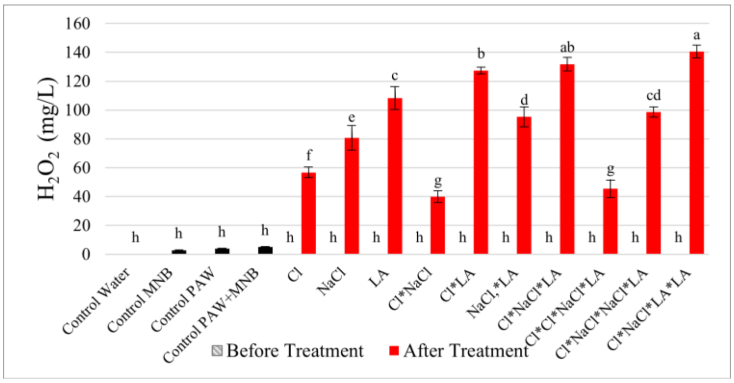


Figure 4. H_2O_2 concentration of chemical solutions using PAW and MNB. Error bars indicate standard deviation (n = 3). Small letters: Same letters indicate no significant difference among treatments (*P* > 0.05), while different letters indicate significant difference among treatments (*P* < 0.05). Abbreviations: PAW = plasma-activated water; MNB = micro/nanobubbles; Cl = Chlorine; NaCl = Sodium Chloride; LA = Lactic Acid

Table 2. The results of H_2O_2 concentration in each ratio of the chemical solutions after treatment with PAW and MNB

Cl (%)	NaCl (%)	LA (%)	Condition	H_2O_2 (mg/L)
100	0	0	Cl	57
0	100	0	NaCl	81
0	0	100	LA	108
50	50	0	Cl*NaCl	40
50	0	50	Cl*LA	127
0	50	50	NaCl*LA	95
33.33	33.33	33.33	Cl*NaCl*LA	132
66.67	16.67	16.67	Cl*CL*NaCl*LA	45
16.67	66.67	16.67	Cl*NaCl*NaCl*LA	99
16.67	16.67	66.67	Cl*NaCl*LA*LA	141

Table 3. **Estimated regression coefficients for H_2O_2 concentration**

Term	Coef	SE Coef	*T*-Value	*P*-Value	VIF
Cl	55.44	4.82	*	*	2.10
NaCl	79.48	4.38	*	*	1.74
LA	106.81	4.38	*	*	1.74
Cl*NaCl	-121.0	23.3	-5.19	0.000	2.34
Cl*LA	175.0	23.3	7.51	0.000	2.34
Cl*Cl*NaCl*LA	-3189	495	-6.44	0.000	3.41
Cl*NaCl*NaCl*LA	2776	488	5.69	0.000	3.31
Cl*NaCl*LA*LA	3172	488	6.50	0.000	3.31

Table 4. **ANOVA for H_2O_2 concentration**

Source	DF	Seq SS	Adj SS	Adj MS	*F*-Value	*P*-Value
Regression	7	34268.5	34268.5	4895.50	70.00	0.000
Linear	2	17152.9	4320.4	2160.22	30.89	0.000
Quadratic	2	8390.6	6736.3	3368.15	48.16	0.000
Cl*NaCl	1	909.5	1885.8	1885.81	26.96	0.000
Cl*LA	1	7481.1	3948.2	3948.22	56.45	0.000
Special Quartic	3	8725.0	8725.0	2908.33	41.58	0.000
Cl*Cl*NaCl*LA	1	105.5	2899.3	2899.28	41.45	0.000
Cl*NaCl*NaCl*LA	1	5663.4	2264.2	2264.16	32.37	0.000
Cl*NaCl*LA*LA	1	2956.1	2956.1	2956.12	42.27	0.000
Residual Error	22	1538.7	1538.7	69.94		
Total	29	35807.2				
R^2 = 95.70%						

Effect of Chemical Solutions with PAW and MNBs on H_2O_2 Concentration

Figure 4 shows the H_2O_2 concentration in the chemical solutions after treatment with PAW and MNBs. The results reveal that the H_2O_2 concentration ranges from 40 to 141 mg/L, representing an average increase of 10 to 20 times compared to the untreated solutions and the control without chemicals. ANOVA analysis was conducted using H_2O_2 concentration data from Table 2. Statistical analysis employed a special quadratic model in a mixture design to examine variable relationships, assess the effects of the independent factors, and predict the response (H_2O_2 concentration). The final results of the mixture regression analysis are shown in Tables 3 and 4.

Table 3 details the coefficients that define the relationship between the ratio of the chemical solutions and the concentration of H_2O_2 using PAW and MNB solutions. All pure solutions have a proportional relationship, while the quadratic and special quartic terms show both positive and negative relationships. The terms with a plus sign (+) mean that an increase in the independent variable results in a rise in H_2O_2 concentration. Conversely, the terms with a minus sign (-) indicate that an increase in the independent variable leads to a decrease in H_2O_2 concentration. For example, increasing the proportion of Cl solution enhances H_2O_2 concentration (as the coefficient of Cl is positive), while mixing Cl and NaCl solutions reduces H_2O_2 concentration (since the Cl*NaCl coefficient is negative).

Additionally, Table 4 presents the ANOVA results for the mixture design of chemical solutions treated with PAW and MNBs. Findings indicate that only the linear terms (Cl, NaCl, and LA), their quadratic terms (Cl*NaCl and Cl*LA), and special quadratic terms (Cl*Cl*NaCl*LA, Cl*NaCl*NaCl*LA, and Cl*NaCl*LA*LA) significantly influence H_2O_2 concentration, as demonstrated by the high *F*-value and a *P*-value <0.05. To evaluate model reliability, the coefficient of determination (R^2) test was applied to the response values obtained from the ANOVA. The R^2 value was 95.70%, indicates that the regression equation can predict the total variance in the H_2O_2 concentration with 95.70% accuracy.

Figure 5 illustrates the importance of residual plots in evaluating linear regression models, as they help assess the quality of the model's predictions. The H_2O_2 concentration data exhibits a patternless distribution, indicating independence and randomness, with points scattered around the horizontal axis without a discernable pattern. This suggests that our linear regression model is a good fit for the data. The normality of the H_2O_2 concentration data was confirmed by a straight-line graph of the results. Furthermore, the variance in H_2O_2 concentration remained stable throughout the experiment, reinforcing the reliability of our predictions.

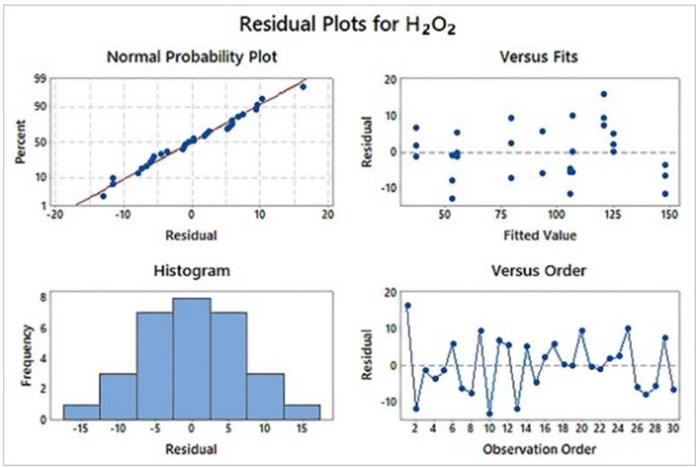


Figure 5. Residual plots for H_2O_2 concentration

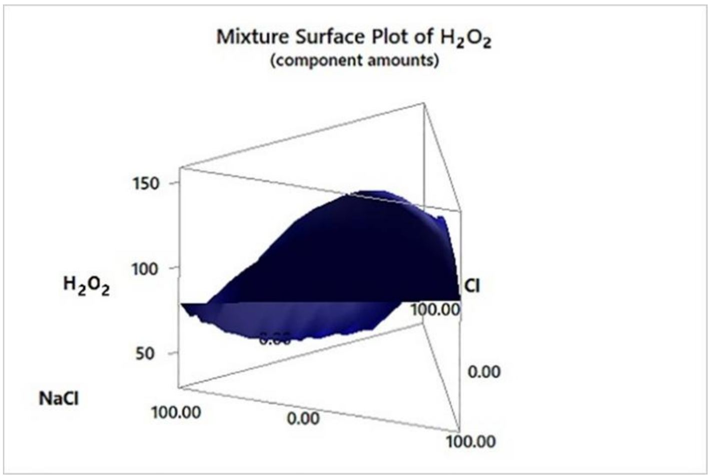


Figure 6. Mixture surface plot of H_2O_2 concentration

The mixture surface plot and contour plot for H_2O_2 concentration in mixed chemical solution is shown in Figures 6 and 7, and illustrates the effects of variable modifications on the response, demonstrating how each component in the mixture influences the H_2O_2 concentration. The results indicate that combining chemical solutions can produce a synergistic effect, leading to an increase in H_2O_2 concentration. This increase is evidenced in Figure 6, where a convex region represents areas of high H_2O_2 concentration. Additionally, Figure 7 shows that the darker blue boundary area corresponds to high H_2O_2 concentration, whereas the lighter blue boundary area indicates lower H_2O_2 concentration.

The optimum conditions for the production of chemical solutions were successfully optimized using PAW and MNBs. This process aims to identify the optimum factors values that bring the response as close as possible to the desired target. By evaluating these factors, we utilized the optimization function in Minitab software to determine the optimum values influencing the maximum concentration of H_2O_2. Additionally, the composite desirability (D) of the appropriate conditions was assessed, with a value ranging from 0 to 1. A value of 0 indicates that one or more factors fall outside acceptable limits, while a value of 1 signifies complete satisfaction with the optimum condition.

Figure 8 illustrates that the graphs of the three chemical solutions exhibit similar trends, initially increasing to a peak and then gradually declining. The peak of each graph represents the maximum H_2O_2 concentration achievable from the chemical solution when treated with PAW and MNBs. Experimental results indicate that the optimal ratio was 17.8% Cl solution, 28.28% NaCl solution,

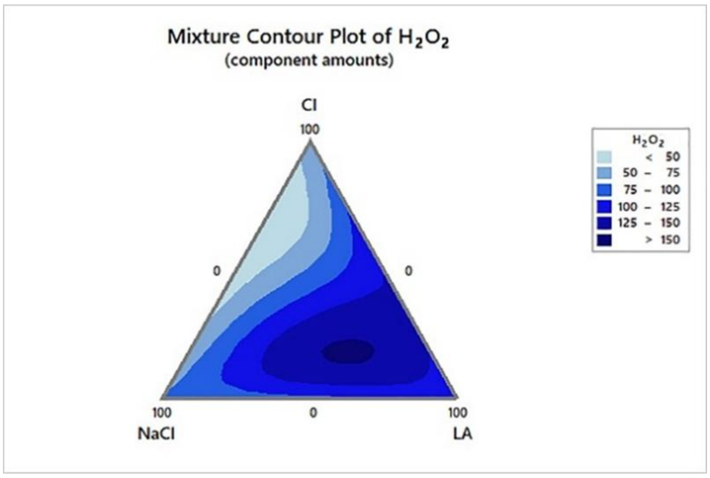


Figure 7. Mixture contour plot of H_2O_2 concentration

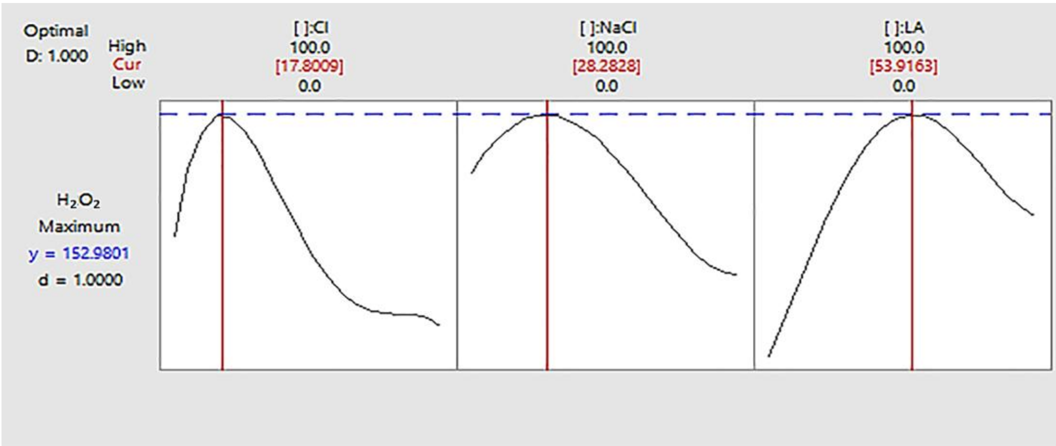


Figure 8. Response optimizer shows the optimal chemical solution ratio that yields the highest H_2O_2 concentration. Abbreviations: Cl = Chlorine; NaCl = Sodium Chloride; LA = Lactic Acid; D = Composite Desirability

and 53.92% LA solution, yielding the highest H_2O_2 concentration of 152.98 mg/L with a D value of 1.000, indicating that this ratio produced the most satisfactory results.

According to the optimal conditions of the proposed model, the H_2O_2 concentration increases as the percentage of LA solution increases and the percentages of Cl and NaCl solutions decrease. The optimum chemical solution ratio predicts a maximum H_2O_2 concentration of 152.98 mg/L. To verify its accuracy, the predicted results were compared with actual data, confirming that the model successfully predicted the H_2O_2 concentration. The actual H_2O_2 concentration obtained with this ratio was 171 mg/L, representing a 12% increase and showing no statistically significant difference from the model prediction.

This research investigated the synergistic effects of chemical solutions containing Cl, NaCl, and LA, treated with PAW and MNB, to enhance physicochemical properties. The examined properties included pH, ORP, EC, and H_2O_2 concentration. To optimize this combined approach, a mixture design was used, varying the concentrations of Cl, NaCl, and LA. The results indicate that PAW- and MNB-activated chemical solutions exhibited a significant decrease in pH and a significant increase in ORP, EC, and H_2O_2 concentration compared to control water. The highest H_2O_2 concentration, 152.98 mg/L, was observed when using PAW and MNB treatment in the chemical solution ratios of Cl (17.80%), NaCl (28.28%), and LA (53.92%). The enhancement of physicochemical properties through the synergistic effects of PAW-and MNB-treated chemical solutions can be explained as follows.

PAW is specially processed water with ROS properties, capable of producing H_2O_2, a highly reactive oxygen-containing compound known for its effectiveness in eliminating pathogens (Pârvulescu *et al*., 2012; Royintarat *et al*., 2020). Research has also revealed the physicochemical properties resulting from the synergy between PAW and MNBs. Tests combining PAW and MNBs showed that the pH of the treated water decreased significantly compared to the MNB-only condition and slightly decreased compared to the PAW-only condition. Additionally, the ORP values increased significantly compared to both the MNB-only and PAW-only conditions. EC values also increased significantly compared to the MNB-only condition and were higher than the PAW-only condition. While H_2O_2 levels increased, the rise was not significant compared to either the MNB-only or PAW-only conditions. This outcome is attributed to the synergistic effects of PAW and MNBs, as PAW generates highly reactive species, a finding consistent with previous studies (Moonsub *et al*., 2022; 2024). Furthermore, introducing MNBs into the system increases the amount of air, leading to more oxygenated water, enhanced surface stability, and extended residence time (Hu and Xia, 2018; Wang *et al*., 2020). These properties allow MNBs to encapsulate reactive species generated by PAW, slowing their decomposition and thereby prolonging their persistence (Moonsub *et al*., 2022). Additionally, MNBs facilitates the rapid transfer of reactive species, effectively disrupting microbial cell walls - an approach that optimizes the efficiency of existing reactors.

In this study, we investigated whether adding chemicals to water and treating it with PAW and MNBs could increase the concentration of H_2O_2. As H_2O_2 levels rose, so did its antimicrobial potential. Furthermore, other properties, such as pH, ORP, and EC, were examined to observe their changing trends. The results demonstrated that the three chemical solutions enhanced the effectiveness of these properties within the synergistic PAW and MNBs as follows:

First, the pH of the chemical solution after treatment with PAW and MNBs significantly decreased compared to the untreated and MNB-only conditions, and slightly increased compared to the PAW-only and PAW combined with MNB conditions. Although the results were similar, the treatment still produced an acidic environment, as several previous studies have reported a decrease in pH after PAW treatment. For example, the pH of untreated water was 6.23, but after treatment, it dropped to 3.87 (Xu *et al*., 2016). The decrease in pH of chemical solutions treated with PAW and MNBs was due to the dissociation of water, air, and chemical molecules energized by PAW and MNBs, leading to the formation of H_2O_2, as described in Equation 8. Additionally, the level of acidity in chemical solutions depended on the type of chemical used. For instance, the pH of Cl and NaCl solutions was slightly lower than that of LA solutions, possibly due to the formation of hypochlorous acid (HOCl) from hypochlorite ions (OCl^-) released in Cl solutions, as described in Equations 10 and 11. In NaCl solutions, HOCl and hydrochloric acid (HCl) formed from chlorine (Cl_2), further increasing acidity, as described in Equations 12 and 13. The LA solution also resulted in a lower pH due to the dissociation and release of protons (H^+). Since H^+ could not exist independently in aqueous solutions, it combined with H_2O to form hydronium ions (H_3O^+), further increasing acidity, as described in Equations 14 and 15. A low pH inhibited microbial growth by disrupting cell structure, causing cell damage, and ultimately leading to microbial death. Previous studies showed that the acidity from PAW reduced microbial counts by 1.6 to 2.3 $\log_{10}$ CFU/mL (Ma *et al*., 2015) and by 1.5 $\log_{10}$ CFU/mL (Xu *et al*., 2016). This decrease in pH also led to an increase in ORP values, indicating a higher oxidation potential (Suslow, 2004).

$$CaCl_2O_2 \rightarrow Ca^{2+} + 2ClO^- \quad (10)$$
$$ClO^- + H_2O \rightarrow HOCl + \cdot OH \quad (11)$$
$$NaCl \rightarrow Na^+ + Cl^- \quad (12)$$
$$Cl_2 + H_2O \rightarrow HOCl + HCl \quad (13)$$
$$C_3H_6O_3 \rightarrow C_3H_5O_3 + H^+ \quad (14)$$
$$H^+ + H_2O \rightarrow H_3O^+ \quad (15)$$

Second, the ORP values of the chemical solutions treated with PAW and MNBs exhibited both increases and decreases compared to the untreated and control conditions. The ORP values of the solutions treated with PAW and MNBs were not significantly different from those of the control water, PAW-only, or MNB-only conditions, but they were significantly lower than the values observed under the PAW combined with MNB conditions. Additionally, solutions containing Cl tended to shift to a more reducing state when treated with PAW and MNBs, resulting in a decrease in ORP. This decrease may have been due to reactions such as chlorine reacting with water to form HOCl, a compound with high oxidizing power. However, HOCl could react with other substances in the water that acted as reductants, donating electrons to these substances and converting HOCl into Cl^-, which significantly lowered oxidizing power, as described in Equation 16 (Harvey, 2019). Because Cl^- was more stable than HOCl, this conversion reduced the disinfection or oxidizing power of the water. Although PAW and MNB treatment decreases the ORP value, it still provides some antimicrobial effects. This is because the treatment removes electrons from microbial cell surfaces, causing cell damage and leakage, ultimately leading to cell rupture and death (Suslow, 2004).

$$HOCl+2e^- \rightarrow Cl^- + OH^- \quad (16)$$

Third, the EC values of chemical solutions treated with PAW and MNBs were higher than those of all control conditions. This increase resulted from the reactive species and ions generated during the treatment process, which significantly altered the EC values. These findings highlight the enhanced synergistic effect of PAW and MNBs treatment on the chemical solutions compared to the controls. Additionally, the NaCl solution exhibited higher electrical conductivity than the Cl and LA solutions, as NaCl is a strong electrolyte. When dissolved in water, NaCl dissociates into Na^+ and Cl^- ions, as described in Equation 12. These free-moving ions contribute to high electrical conductivity. Therefore, any condition containing NaCl solution will increase the EC values. However, when treated with PAW and MNBs, the Na^+ and Cl^- ions can react with other compounds, reducing the ion concentration and leading to a lower EC value compared to the untreated condition. Previous studies have reported increased EC values after PAW treatment, with EC rising from 17 μS/cm in the control group to 218 μS/cm (Xu *et al*., 2016) and up to 450 μS/cm (Ma *et al*., 2015). However, our method resulted in EC values increased several times more than in previous methods. Finally, the concentration of H_2O_2, a reactive oxygen species (ROS) produced through PAW activation, as described in Equation 8, has been explored in previous reports. Some studies suggest that H_2O_2 formation involves the combination of two ·OH radicals generated by the dissociation of water and air molecules (Gorbanev *et al*., 2016; Lukes *et al*., 2008), as outlined in Equations 7 and 1, respectively. These ·OH radicals exhibit a strong pathogen-killing effect. The results revealed that chemical solutions treated with PAW and MNBs significantly increase H_2O_2 concentration, with an average rise of 10 to 20 times compared to conditions with PAW alone, MNBs alone, and the combination of PAW with MNBs. This increase in H_2O_2 concentration was attributed to the dissociation of chemical solutions, as described in Equations 10-18. Statistical analysis revealed a correlation between the solubility of the chemicals and the concentration of H_2O_2 in PAW and MNB solutions. Conditions with positive coefficients had the potential to generate a large amount of ·OH radicals, leading to increased H_2O_2 formation, a finding consistent with previous studies reporting higher H_2O_2 concentrations in solutions containing PAW and MNBs (Hänsch *et al*., 2015; Qian *et al*., 2020). Conversely, conditions with negative coefficients showed an antagonistic relationship with the H_2O_2 concentration, producing fewer ·OH radicals, thereby reducing H_2O_2 formation. Additionally, the conditions of Cl**LA, Cl*NaCl*LA, and Cl*NaCl*LA*LA* did not exhibit significant differences in their effects. The LA solution appeared to have a greater impact on H_2O_2 concentration compared to other solutions, as it releases a substantial amount of H^+ when combined with oxygen, as in Equation 14, which enhances H_2O_2 formation. Moreover, adjusting the pH of the LA solution with NaOH to minimize the influence of protons increases the availability of OH^- ions, as in Equation 17, further promoting H_2O_2 formation. Although Cl and NaCl solutions also contribute to H_2O_2 concentration, they yield lower amounts compared to the LA solution. In the Cl solution, the increase in H_2O_2 results from the release of OH^- ions, as described in Equations 10 and 11. Similarly, in NaCl solutions, H^+ ions react with oxygen, as described in Equations 12 and 19, leading to H_2O_2 formation. These findings indicate that a solution with 16.67% Cl, 16.67% NaCl, and 66.67% LA produced the highest H_2O_2 concentration of 141 mg/L. For optimal efficiency, the effective chemical ratio was determined to be 17.8% Cl, 28.28% NaCl, and 53.92% LA, yielding the highest H_2O_2 concentration of 152.98 mg/L. This data suggests that the LA solution significantly influences H_2O_2 concentration, while Cl and NaCl play a lesser role. The chemical solution prepared with this ratio was combined with PAW and MNBs, where the energy from PAW and MNBs stimulated the decomposition of water, oxygen, and the chemical solution, leading to an increase in H_2O_2 concentration.

$$NaOH + H_2O \rightarrow Na^+ + H^+ + 2\cdot OH \quad (17)$$
$$Cl^- + H_2O \rightarrow HOCl + H^+ \quad (18)$$

Our results are consistent with previous reports highlighting the potential of using PAW alone. For example, treating DI water with PAW at 8 kV, 2.5 A, and 12 Hz increased H_2O_2 concentrations from 0.49-0.51 mg/L to 1.63-1.76 mg/L (Royintarat *et al*., 2019a). Another study reported that treating DI water with PAW at 750 W resulted in H_2O_2 concentrations ranging from 17-20 μmol/L (Xiang *et al*., 2019). Additionally, our findings align with studies emphasizing the synergistic potential of combining PAW and MNBs. Treating DI water with PAW at 30 W and MNBs at 3 bar increased H_2O_2 concentrations to 5.34 mg/L and 14.56 mg/L (Moonsub *et al*., 2022; 2024). These previous studies reported lower H_2O_2 concentrations than our study, which achieved a maximum H_2O_2 concentration of 152.98 mg/L, suggesting that the synergistic efficiency can be further improved. The combination of PAW, MNBs, and chemical solutions demonstrates strong potential for increasing H_2O_2 concentrations. It is widely accepted that high concentrations of H_2O_2 are effective as an oxidizing agent and are commercially available in aqueous solutions across a broad concentration range (Eul *et al*., 2001). This agent can eliminate microorganisms from objects and product surfaces, thereby preserving quality during storage. A related study investigated the use of concentrated H_2O_2 for antimicrobial treatment of cherry tomatoes. The results showed a significant reduction in bacteria, coliforms, yeasts, and molds, extending the tomatoes' shelf life up to 18 days (Islam *et al*., 2018). Previous research has highlighted the potential of H_2O_2 to eliminate or inhibit microorganisms. Our study, which leverages the synergy of PAW, MNBs, and chemical solutions, yields higher concentrations of H_2O_2 with unique properties compared to those commonly used in industry. These properties include high purity, fine particle size, high energy, self-degradability, and lack of residue, making this an environmentally friendly, chemical-free technology. These characteristics enhance its effectiveness in eliminating or inhibiting microorganisms. This method is well-suited as an antimicrobial agent in food applications, representing an innovative approach for the food industry in terms of food safety and healthcare.

Conclusions

This research focused on studying the synergistic of chemical solutions consisting of Cl, NaCl, and LA, treated with PAW and MNBs to enhance physicochemical properties. Specifically, the study investigated changes in pH, ORP, EC, and H_2O_2 concentration. The experimental results showed that the chemical solutions with PAW and MNBs led to a decrease in pH compared to the untreated and MNB-only conditions, with a slight increase compared to the PAW-only and PAW combined with MNB conditions. ORP values were not significantly different from those of control water, PAW-only, or MNB-only conditions but were significantly lower than those observed under the PAW combined with MNB condition. EC values showed a significant increase compared to all controls. H_2O_2 concentration, the primary indicator of antimicrobial potential, was significantly higher than in the untreated solution and all controls. The ANOVA revealed a significant effect on H_2O_2 concentration. Further research aimed at determining the optimal chemical solution ratio found that the most effective combination for PAW and MNB treatment consisted of 17.80% Cl, 28.28% NaCl, and 53.92% LA. This ratio predicted a maximum H_2O_2 concentration of 152.98 mg/L, while experimental data showed an actual concentration of 171 mg/L, a 12% increase, though not statistically significant different from the model's prediction. These results indicate that combining chemical solutions with PAW and MNBs enhances the H_2O_2 concentration, thereby increasing antimicrobial potential. This approach presents a novel and effective method for controlling microorganisms in the food industry, ultimately enhancing food safety. Further studies are needed to evaluate the impact of this combination on microbial inhibition.

Acknowledgement

This research received financial support from Advanced Technology and Innovation Management for Creative Economy Research Group, Faculty of Engineering, Chiang Mai University. The authors also wish to thank the Department of Industrial Engineering, Faculty of Engineering, Division of Biotechnology, Faculty of Agro-Industry, Plasma and Beam Physics Research Facility, Department of Animal and Aquatic Sciences, Faculty of Agriculture, Chiang Mai University, for providing research facilities.

References

Agarwal, A., Ng, W.J., and Liu, Y. (2011). Principle and applications of microbubble and nanobubble technology for water treatment. Chemosphere, 84(9):1175-1180. https://doi.org/10.1016/j.chemosphere.2011.05.054

Bai, Y., Muhammad, A.I., Hu, Y., Koseki, S., Liao, X., Chen, S., Ye, X., Liu, D., and Ding, T. (2020). Inactivation kinetics of *Bacillus cereus* spores by Plasma activated water (PAW). Food Research International, 131:109041. https://doi.org/10.1016/j.foodres.2020.109041

Ben-Yehoshua, S. and Mercier, J. (2005). UV irradiation, biological agents, and natural compounds for controlling postharvest decay in fresh fruits and vegetables. In: Environmentally Friendly Technologies for Agricultural Produce Quality. Singh, B.K., Thomson, J.F., and Whiteman, N.K. (Eds.), CRC Press, p. 265-299. https://doi.org/10.1201/9780203500361.sec2

Choi, M.-S., Jeon, E.B., Kim, J.Y., Choi, E.H., Lim, J.S., Choi, J., and Park, S.Y. (2022). Application of dielectric barrier discharge plasma for the reduction of non-pathogenic

Escherichia coli and *E. coli* O157:H7 and the quality stability of fresh oysters (*Crassostrea gigas*). LWT, 154:112698. https://doi.org/10.1016/j.lwt.2021.112698

Darmanin, M., Kozak, D., de Oliveira Mallia, J., Blundell, R., Gatt, R., and Valdramidis, V.P. (2020). Generation of plasma functionalized water: Antimicrobial assessment and impact on seed germination. Food Control, 113:107168. https://doi.org/10.1016/j.foodcont.2020.107168

Eul, W., Moeller, A., and Steiner, N. (2001). Hydrogen peroxide. In: Kirk-Othmer Encyclopedia of Chemical Technology, (Ed.), John Wiley and Sons. https://doi.org/10.1002/0471238961.0825041808051919.a01.pub2

Fan, W., Cui, J., Li, Q., Huo, Y., Xiao, D., Yang, X., Yu, H., Wang, C., Jarvis, P., Lyu, T., and Huo, M. (2021). Bactericidal efficiency and photochemical mechanisms of micro/nano bubble–enhanced visible light photocatalytic water disinfection. Water Research, 203:117531. https://doi.org/10.1016/j.watres.2020.117531

Francis, G.A. and O'Beirne, D. (2005). Variation among strains of *Listeria monocytogenes*: differences in survival on packaged vegetables and in response to heat and acid conditions. Food Control, 16(8):687-694. https://doi.org/10.1016/j.foodcont.2004.06.005

Gorbanev, Y., O'Connell, D., and Chechik, V. (2016). Non-thermal plasma in contact with water: The origin of species. Chemistry – A European Journal, 22(10):3496–3505. https://doi.org/10.1002/chem.201503771

Graça, A., Abadias, M., Salazar, M., and Nunes, C. (2011). The use of electrolyzed water as a disinfectant for minimally processed apples. Postharvest Biology and Technology, 61(2–3):172–177. https://doi.org/10.1016/j.postharvbio.2011.04.001

Hänsch, M.A.C., Mann, M., Weltmann, K.-D., and von Woedtke, T. (2015). Analysis of antibacterial efficacy of plasma-treated sodium chloride solutions. Journal of Physics D: Applied Physics, 48(45):454001. https://doi.org/10.1088/0022-3727/48/45/454001

Harvey, D. (2019). P2: Standard reduction potentials by value. DePauw University [Internet].

Hu, L. and Xia, Z. (2018). Application of ozone micro-nano-bubbles to groundwater remediation. Journal of Hazardous Materials, 342:446–453. https://doi.org/10.1016/j.jhazmat.2017.08.030

Islam, M.Z., Mele, M.A., Hussein, K.A., and Kang, H.-M. (2018). Acidic electrolyzed water, hydrogen peroxide, ozone water and sodium hypochlorite influence quality, shelf life and antimicrobial efficacy of cherry tomatoes. Research Journal of Biotechnology, 13(4):51-55.

Jyung, S., Kang, J.W., and Kang, D.H. (2023). Inactivation of Listeria monocytogenes through the synergistic interaction between plasma-activated water and organic acid. Food Research International, 167:112687. https://doi.org/10.1016/j.foodres.2023.112687

Lukes, P., Clupek, M., Babicky, V., and Sunka, P. (2008). Ultraviolet radiation from the pulsed corona discharge in water. Plasma Sources Science and Technology, 17(2):024012. https://doi.org/10.1088/0963-0252/17/2/024012

Ma, R., Wang, G., Tian, Y., Wang, K., Zhang, J., and Fang, J. (2015). Non-thermal plasma-activated water inactivation of food-borne pathogen on fresh produce. Journal of Hazardous Materials, 300:643-651. https://doi.org/10.1016/j.jhazmat.2015.07.061

Mai-Prochnow, A., Zhou, R., Zhang, T., Ostrikov, K., Mugunthan, S., Rice, S.A., and Cullen, P.J. (2021). Interactions of plasma-activated water with biofilms: Inactivation, dispersal effects and mechanisms of action. NPJ Biofilms and Microbiomes, 7:11. https://doi.org/10.1038/s41522-021-00191-6

Moonsub, K., Boonyawan, D., Tonglek, V., and Wattanutchariya, W. (2022). Enhancing efficiency of plasma-activated water using microbubbles/nanobubbles techniques. In: Proceedings of the 12th Annual International Conference on Industrial Engineering and Operations Management. March 7-10, 2022, Istanbul, Turkey, p. 1080-1088.

Moonsub, K., Seesuriyachan, P., Boonyawan, D., and Wattanutchariya, W. (2024). Synergistic effect of plasma-activated water with micro/nanobubbles, ultraviolet photolysis, and ultrasonication on enhanced Escherichia coli inactivation in chicken meat. Processes, 12(3):567. https://doi.org/10.3390/pr12030567

Oh, J.-S., Olabanji, O.T., Hale, C., Mariani, R., Kontis, K., and Bradley, J.W. (2011). Imaging gas and plasma interactions in the surface-chemical modification of polymers using micro-plasma jets. Journal of Physics D: Applied Physics, 44(15):155206. https://doi.org/10.1088/0022-3727/44/15/155206

Pârvulescu, V.I., Magureanu, M., and Lukes, P. (2012). Plasma Chemistry and Catalysis in Gases and Liquids. 1st ed. Wiley-VCH Verlag GmbH & Co. KGaA. https://doi.org/10.1002/9783527649525

Qian, J., Wang, C., Zhuang, H., Zhang, J., and Yan, W. (2020). Oxidative stress responses of pathogen bacteria in poultry to plasma-activated lactic acid solutions. Food Control, 118:107355. https://doi.org/10.1016/j.foodcont.2020.107355

Ramos, B., Brandao, T.R.S., Teixeira, P., and Silva, C.L.M. (2020). Biopreservation approaches to reduce *Listeria monocytogenes* in fresh vegetables. Food Microbiology, 85:103282. https://doi.org/10.1016/j.fm.2019.103282

Rothwell, J.G., Alam, D., Carter, D.A., Soltani, B., McConchie, R., Zhou, R., Cullen, P.J., and Mai-Prochnow, A. (2022). The antimicrobial efficacy of plasma-activated water against Listeria and E. coli is modulated by reactor design and water composition. Journal of Applied Microbiology, 132(4):2490–2500. https://doi.org/10.1111/jam.15429

Royintarat, T., Choi, E.H., Boonyawan, D., Seesuriyachan, P., and Wattanutchariya, W. (2020). Chemical-free and synergistic interaction of ultrasound combined with plasma-activated water (PAW) to enhance microbial inactivation in chicken meat and skin. Scientific Reports, 10:1559. https://doi.org/10.1038/s41598-020-58199-w

Royintarat, T., Choi, E.H., Seesuriyachan, P., and Wattanutchariya, W. (2019a). Ultrasound-assisted plasma-activated water for bacterial inactivation in poultry industry. In: Proceeding of the IEEE International Conference on Industrial Technology (ICIT), February 13–15, 2019, Melbourne, Australia, p. 1028–1032. https://doi.org/10.1109/ICIT.2019.8755067

Royintarat, T., Seesuriyachan, P., Boonyawan, D., Choi, E.H., and Wattanutchariya, W. (2019b). Mechanism and optimization of non-thermal plasma-activated water for bacterial inactivation by underwater plasma jet and delivery of reactive species underwater by cylindrical DBD plasma. Current Applied Physics, 19(9):1006–1014. https://doi.org/10.1016/j.cap.2019.05.020

Selma, M.V., Allende, A., Lopez-Galvez, F., Conesa, M.A., and Gil, M.I. (2008). Disinfection potential of ozone, ultraviolet-C and their combination in wash water for the fresh-cut vegetable industry. Food Microbiology, 25(6):809–814. https://doi.org/10.1016/j.fm.2008.04.005

Sruthi, N.U., Josna, K., Pandiselvam, R., Kothakota, A., Gavahian, M., and Khaneghah, A.M. (2022). Impacts of cold plasma treatment on physicochemical, functional, bioactive, textural, and sensory attributes of food: A comprehensive review. Food Chemistry, 368:130809. https://doi.org/10.1016/j.foodchem.2021.130809

Suslow, T.V. (2004). Oxidation-reduction potential (ORP) for water disinfection monitoring, control, and documentation

[Internet]. University of California Agriculture and Natural Resources. https://doi.org/10.3733/ucanr.8149

Thirumdas, R., Kothakota, A., Annapure, U., Siliveru, K., Blundell, R., Gatt, R., and Valdramidis, V.P. (2018). Plasma activated water (PAW): Chemistry, physicochemical properties, applications in food and agriculture. Trends in Food Science and Technology, 77:21–31. https://doi.org/10.1016/J.TIFS.2018.05.007

Velázquez, L.D.C., Barbini, N.B., Escudero, M.E., Estrada, C.L., and de Guzmán, A.M.S. (2009). Evaluation of chlorine, benzalkonium chloride and lactic acid as sanitizers for reducing *Escherichia coli* O157:H7 and *Yersinia enterocolitica* on fresh vegetables. Food Control, 20(3): 262–268. https://doi.org/10.1016/j.foodcont.2008.05.012

Wang, S., Liu, Y., Li, P., Wang, Y., Yang, J., and Zhang, W. (2020). Micro-nanobubble aeration promotes senescence of submerged macrophytes with low total antioxidant capacity in urban landscape water. Environmental Science: Water Research and Technology, 6(3):523–531. https://doi.org/10.1039/C9EW00929A

Xiang, Q., Liu, X., Liu, S., Ma, Y., Xu, C., and Bai, Y. (2019). Effect of plasma-activated water on microbial quality and physicochemical characteristics of mung bean sprouts. Innovative Food Science and Emerging Technologies, 52:49–56. https://doi.org/10.1016/j.ifset.2018.11.012

Xu, Y., Tian, Y., Ma, R., Liu, Q., and Zhang, J. (2016). Effect of plasma activated water on the postharvest quality of button mushrooms, *Agaricus bisporus*. Food Chemistry, 197(Part A):436–444. https://doi.org/10.1016/j.foodchem.2015.10.144