Enhancing Efficiency of Plasma-Activated Water Using Microbubbles/Nanobubbles Techniques

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Abstract

This research investigated the treatment of Plasma-Activated Water (PAW) and Microbubbles/Nanobubbles (MNB) to increase the disinfection efficiency. The sequence of PAW and MNB preparation, as well as the type of water, were studied, investigated four operating conditions by comparing the sequence of MNB (before PAW or concurrent with PAW) at two types of water (RO or tap water). Plasma activated water characterized the effectiveness of PAW/MNB in terms of chemical properties, consisting of EC pH ORP and H_2O_2 concentration. The measurements of EC showed that the treatment with MNB before PAW on tap water produced the highest amount of EC, which is 222.33 μS/cm. The pH measurements showed the acidic pH in MNB incorporating PAW studies ranged from 3.24 to 4.32. In the meantime, the treatment MNB before PAW on tap water also led to the highest amount of ORP, which is 492.70 mV. Finally, the H_2O_2 measurement showed that treated water with MNB before PAW could induce the highest H_2O_2 concentration, 6.13 mg/L. This study concludes that the sequence of MNB and PAW treatment and the water type have influenced EC in Tap and RO water, ORP in RO water, and H_2O_2 content in Tap and RO water. Further study based on this result will further investigate foodborne pathogens in the Poultry Industry.

Keywords
Plasma Technology, Microbubbles/nanobubbles, hydrogen peroxide, foodborne pathogens, Poultry Industry

1. Introduction
The poultry industry is an industry that generates income and continues to grow in Thailand. Based on the Food and Agriculture Organization forecasts, The United Nations (FAO) provides an overview of the poultry industry, found in the OECD-FAO Agricultural Outlook 2016 - 2025 report, which analyzes the future of the world livestock situation. The meat market is still strong; cereal prices are still as low as before. The cost of animal feed has always fluctuated

during the past decade; in 2025, the world's meat production tends to increase by 16% compared to 2013 - 2015. It expects that most meat production increases in developing countries, all using more concentrated protein in livestock food. Poultry meat is the primary driver of product growth. Making poultry meat an option for producers and consumers in developing countries, consuming meat globally per person/year Expected to increase 35.3 kilograms by 2025, which is an increase of 1.3 kilograms per day. However, the biggest concern in Poultry is food safety due to the activities of endogenous microbes and enzymes, causing rotten food to lose quality and have a limited shelf life. Many companies used chemicals for disinfection, but this method caused residue, leading to consumer health problems. However, due to the increase in fresh chicken meat, it is necessary to develop a storage method that is highly effective against microbes. Safe to use to maintain food quality that affects consumer health. From the stated problems, many researchers have investigated and shown that plasma technology could reduce pathogens in chicken meat with reactive oxygen species (ROS) and the reactive nitrogen species (RNS) that occur during the plasma process. ROS causes damage to DNA proteins and enzymes that cause cell damage. ROS also causes lipid peroxidation and amino acid oxidation in the cell membrane. Therefore, it is an integral part of destroying microbes efficiently (Royintarat et al. 2018). PAW is an implementation of plasma technology that has been implemented for the disinfection of fresh chicken meat in the prior study (Royintarat et al. 2017). However, in the production process that the chicken meat is thick, complex, or has fat in the system, it will reduce the sterilization efficiency. Therefore, more research is needed to increase the efficiency of killing germs in chickens. The researcher then proposed a method to increase sterilization efficiency by combining PAW with MNB to increase the efficiency of disinfection of chicken meat and evaluate optimal production parameters that could contribute to disinfecting pathogens in the poultry industry.

1.1 Objectives
This study aims to analyze the effect and evaluate the appropriate condition of PAW combined with MNB technology for the inactivation of foodborne pathogens in Poultry.

2. Literature Review
2.1 Plasma microorganism inhibition mechanisms
A clear explanation of inactivation mechanisms is a significant issue in applying plasma technology to attain success in practice. Numerous studies have been conducted to date on the implementation of NTP to inhibit microorganisms (Liao et al. 2017). The research discovered that the primary mechanism could be split into biological features.

1) DNA damage by UV radiation
In the first, DNA damage directly induced by plasma-produced UV rays was suggested as a microbial inactivation mechanism (Boudam et al. 2006). However, it is well established that UV light, particularly for a period of approximately 260 nm, can trigger the response of thymine and cytosine in the same DNA fiber as a dimer and render it capable of replicating.

2) Lipid Peroxidation
Oxidation damage to cell membranes or cells (intracellular components) (such as DNA, proteins, carbohydrates, etc.) is a possible mechanism for murdering NTP (Non-Thermal Plasma) organisms among these components. Membrane lipid, particularly polyunsaturated fatty acids PUFA, is the weakest component owing to its proximity to the cell surface and sensitivity to the oxygen species (ROS) response. (Alkawareek et al. 2014). During the fat oxidation chain response (lipid peroxidation chain response), the H atom is obtained from PUFA by ROS from fatty acid radical (L). It is oxidized to lipid hydroperoxide (LOOH) by O_2 (Liao et al. 2017).

Barrier-discharge (FE-DBD) induces lipid peroxidation in the cell membrane. *E. Coli* and lipid peroxides, such as malondialdehyde (MDA), can damage DNA and proteins. The formation of covalent adducts (Del Rio et al. 2005). Plasma-caused ROS species not only directly influence the cell membrane but also through the outer membrane of the cell parts (Liao et al. 2017). For instance, Han et al. (2015) studied various kinds of atmospheric pressure plasma that influence microorganisms Gram-positive and Gram-negative. They showed that for Gram-nagative, such as *E. Coli* , ROS affected the lipid membrane through peroxidative responses. On the other hand, for *S. Aureus* with Gram-positive, ROS may be transferred through the cell membrane and cause oxidative damage to the intracellular membrane. Another observation for harm induced by NTP may not be necessary to achieve cell death relative to a large quantity of internal stress, showing that the cause of the bacteria is still alive. But in a state that cannot develop a nonculturable condition (VBNC), many trials indicate that short-term exposure to plasma may cause bacteria in the VBNC state. (Abramzon et al. 2006). Dolezalova and Lukes (2015) have reported that *E.coli* suspension with a non-thermal atmospheric pressure plasma (APPJ) device with a decrease of 7.0 logs stating that most cells cause VBNC after therapy Plasma. It was discovered the marker of lipid peroxidation was a mechanic.

3) Protein Modulation

The plasma effect adjustment of protein structure by microbial inhibition mechanism (Lioa et al. 2017) has also been identified. Study using SDS-PAGE and 2D PAGE to demonstrate that the modification and chemical degradation of membrane proteins occurs after exposure to plasma, which may be induced by hydroxyl radicals caused by plasma. On the other side, the protein surface of the cell was discovered to have changed after plasma therapy.

4) Inducing Apoptosis

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 PCM cell death. In mammalian cells (Ahn et al. 2011). In addition to enhanced research, PCD can also be shown to happen. In bacteria (Bayles 2014). Plasma may be linked to the PCD, the bacterial mechanism by preventing the plasma. Based on studies conducted by Li et al. (2015), the suggested apoptosis death mechanism impacts the inhibiting impact of DBD plasma against Microcystis aeruginosa through intracellular plasma ROS development.

Inside the cell, Besides, studies by Lunov et al. (2016) demonstrate that short-term exposure of plasma DBD (15 seconds) leads to a physiological sign of apoptosis-like mortality. The bacteria show that NTP may cause program-specific bacterial cell death (PCD). Also, the high voltage and low voltage plasma DBD, which was handled for 15 seconds, triggered contact with phosphatidylserine. On the cell surface and causes the expression of the character that is similar to Pseudomonas aeruginosa, *E. coli*, *S. aureus*, and *B. subtilis*. Therefore, the short-term disinfection technique of plasma causes the accumulation of intracellular ROS to cause the death of Apoptosis in bacteria in cells. Therefore, more attention should be paid to the impact of plasma in the future.

According to research conducted by Kim et al. (2015) in this study, the Pressure Corona Discharge Plasma Jet (CDPJ) is used to eliminate microbial contamination of certain emulsions, which is commonly used on Korean rice rolls ready to eat. Gimbap (Kimbab) found microbial pollutants on the surface of the industrial. L drying pads, such as aerobic bacteria, fungi, and marine bacteria. At 20 output pressure CDPJ is created. DC kV and 58 kHz peak. When exposed to CDPJ, the aerobic living cells were reduced for 20 minutes by more than 2 logs (99 percent). In conclusion, CDPJ three is Used for food surface contamination without affecting the food's physical properties.

The atmospheric pressure cold plasma technology for microbiological contamination of foods and biological materials was studied (Mok et al. 2014). In this study, the most common foodborne pathogens were suppressed by Afterglow Corona Discharge Air Plasma (ACDAP): Escherichia coli, Staphylococcus aureus, Salmonella typhimurium, Bacillus cereus, Listeria monocytogenes. Plasma corona discharge is solid. Output pressure at 20 kV DC and 58 kHz frequency, centrifugal blower supplying 2.5 m/s of electrode-level wind speed, is used for generation of plasma currents. The temperature rises to around 20°C during plasma exposure, and the relative humidity in the laboratory is decreased by 50 percent within 3 hours. The experiment showed that specific foodborne pathogens were reduced to 3.5-log (99.97 percent) in counting the number of living pathogens cells measured in food, in particular, Escherichia coli O157: H7, contained 24 hours a day. The pathogen inhibition form is better described. Singh-Heldman In conclusion, the ACDAP shows it effectively inhibits common foodborne pathogens.

Consistent with the research of Royintarat et al. (2018), a study of the This research generated PAW using three different techniques: dielectric barrier discharge plasma jet with radio frequency (RF) power supply, plasma jet with flyback transformer (FBT), and pin-to-pin discharge in water with a high-voltage (HV) power supply. Input variables of power, gas ratio, gas flow, electrode distance, and exposure time were varied to compare their effects on pH, oxidation-reduction potential (ORP), electrical conductivity (EC), hydrogen peroxide (H_2O_2), and bacteria reduction. The study used a design of experiment (DOE) technique optimized by a response surface method and a multiple-response desirability function. The optimal conditions for RF, FBT, and HV sources reduced Escherichia coli by 1.79, 2.23, and 3.03 log, respectively, and Bacillus cereus by 0.01, 0.03, and 1.77 records, respectively. In addition, the H_2O_2 that was produced could be stored for approximately 9, 10, and 6 d, respectively.

2.2 Plasma-Activated Water (PAW) and Micro/Nanobubble technique

From the research of Sritontip et al. (2019), the study of seed germination stimulation Physiological development in plants with high voltage plasma (HVP) and air fine (micro/nano) bubbles FBs). This study studied Chinese coriander (Apium graveolens L.) and sweet corn (Zea mays L). A second experiment followed to explore further the effect of FBs on the growth and quality of fruits in melon (Cucumis melo L.). The results suggest that all treatments of HVP + FBs, HVP, and FBs help. Increase the germination rate of Chinese celery seeds and sweet corn varieties compared to using normal distilled water. It also found that HVP and FBs in the treatment lead to the most extended root growth. For sweet corn seedlings, HVP + FBs, HVP, and FBs can increase the root length. Also, melon experiments show that FBs can improve leaf width, height, and number and increase the number of flowers per vine compared to untreated plants. However, the quality of the results was similar. While the leaves, stem, and total dry matter increased weight in FBs. From the inhibition of bacterial growth, HVP + FBs inhibited the growth of bacteria in plants. Therefore, it can be concluded that HVP + FBs can stimulate seed germination and effectively inhibit bacterial growth. The generation of highly reactive free radicals and turbulence associated with collapsing MBs has great potential for water disinfection. Hydrodynamic cavitation is a cost-effective method for water disinfection compared with acoustic cavitation. However, laboratory-scale research indicates hydrodynamic cavitation costs for water disinfection are still higher than traditional chlorination and ozonation (Jyoti and Pandit, 2001). Under different conditions, the impact of ozone MBs on Escherichia coli was investigated. The more efficient disinfection kinetics of *E.coli* has been found. Ozone MBs were found, resulting in reduced reactor size and limited ozone dose compared to traditional ozone disinfection for the same 2-log inactivation disinfection efficiency (Sumikura et al. 2007). The radical and shock waves produced by the collapse of MBs were considered the leading cause of coliform inactivation in this process. However, the exact contribution of each impact to coliform inactivation remains unknown. In addition, high productivity for inactivation of *E. coli* MBs created by hydrodynamic cavitation. It is also produced in water disinfection (Jyoti and Pandit 2001). The use of high frequency, low power ultrasound, and MBs has shown great potential in controlling bacteria and algae attachment onto solid surfaces (Broekman et al. 2010).

3. Methods

3.1. Study information and related theories

Studying practical considerations for enhancing efficiency in Plasma Technology and supplementary disinfection techniques needs to have theoretical understanding and review associated studies on significant problems such as common foodborne pathogens, PAW and MNB technology.

3.2 Experiment and analyze the factors of PAW and MNB

In the study of the experiment and analysis of PAW and MNB technique, the experiment was intended to assess the impact of plasma on enhancing efficiency. The response is the amount of common foodborne pathogens. Therefore, the target value is the decrease of common foodborne pathogens in the poultry industry. The experiment used PAW with flyback transformer (FBT) Power 30 Watt for 20 min at 25°C. In the investigation, the optimal conditions for FBT source Plasma activated water (PAW) can generate H_2O_2 concentration levels in the ranges of 0.5–1.68 ppm. The optimum selected values can inactivate foodborne pathogens (Royintarat et al. 2018). MNB condition used the pressure of 3 bar for 5 min at 25 °C from preliminary study. The experiment condition is shown in Table1**.** A total of 10 experiments were conducted: Use MNB first and then PAW on tap water and RO water, use MNB concurrent with PAW on tap water and RO water, Control PAW on tap water and RO water, Control MNB on tap water and RO water, Control water on tap water and RO water respectively. Each experiment was repeated three times. Then, the treated water was characterized in terms of EC pH ORP and H_2O_2 concentration and programmatically evaluated Turkey Pairwise Comparisons One-way ANOVA by Minitab® Statistical Software © 2021 Minitab, LLC.

4. Results and Discussion

The research has intended to test the efficiency of PAW combined with MNB techniques. According to the results, the measurements of EC show that treatment water with MNB first and then PAW on tap water has the highest amount of EC, which is 223.33 μS/cm, as illustrated in Figure 1. The pH measurements show that combining MNB with PAW leads to the acidic pH of the water, which ranges from 3.24-4.32 shown in Figure 2. Due to the presence of PAW-generated ROS, an acidic radical in the experiment. Furthermore, the measurements of ORP show that the treated water with MNB before PAW on tap water causes the highest ORP, which is 492.70 mV, as shown in Figure 3. Comparing the PAW and NMB use sequences, the experiment showed that the EC and ORP values in the MNB concurrent with the PAW condition were lower than those of the MNB before the PAW condition. Due to the NMB trap radical in the bubble, electrical conductivity and oxidation-reduction were detected low potential in the system. Finally, the measurement of H_2O_2 concentration, as shown in Figure 4, also indicates that treating water with MNB before PAW on tap water leads to the highest H_2O_2 concentration, which is 6.13 mg/L, as shown in Table 1.

Then, the researcher used the Minitab software to analyze the results of the statistical experiments to see whether the main factors (MNB/PAW sequence and water type) had a statistically significant impact at the statistical level. The factor or interaction pair had a statistically significant effect if it was smaller than the level of $\alpha = 0.05$. The EC analysis of Tap and RO water shows the p-values of 0.08 and 0.03, respectively, in the sequence trials, showing that the order in which PAW and MNB were used had a significant effect on EC. Use MNB before using PAW-generated water to carry electrical current, resulting in a more significant EC value in the solution. The water type comparison experiment revealed that the water type significantly affects the EC value, with the p-values of 0.018 and 0.008 for Tap/RO water, respectively. With higher conductivity of tap water than RO water, tap water represents a higher value of EC in this study.

On the other hand, the statistical analysis on pH shows that p-values were more than 0.05, concluding that the order of PAW and MNB and the water type had no significant effect on pH. Furthermore, the ORP analysis of Tap and RO water has p-values larger than 0.05, showing that water type does not affect the ORP value. However, the p-value of the ORP test from the sequence of MNB and PAW is less than 0.5, showing that treating water with MNB before PAW is significantly larger than the concurrent treatment. Finally, the statistical analysis of H_2O_2 concentration on the sequence of treatment and water type has p-values less than 0.05, showing that both factors affect the amount of H_2O_2 attention in the experiment.

4.1 Numerical Results

Table1. Test results for PAW and MNB on Chemical properties

Experiment		pH		EC (μS/cm)		ORP (mV)		H_2O_2 (mg/L)	
		Mean	SD	Mean of	SD	Mean	SD	Mean	SD
MNB before PAW	Tap water	3.24	0.02	222.33	2.52	492.70	1.81	6.13	0.4
	RO water	3.60	0.3	196.00	2.0	454.80	3.4	5.34	0.1
MNB concurrent with PAW	Tap water	4.32	0.1	115.00	2.0	354.40	2.75	5.14	0.2
	RO water	3.50	0.4	98.00	3.2	343.90	3.1	4.77	0.3
Control PAW	Tap water	4.02	0.1	137.70	2.5	394.60	4.88	4.05	0.2
	RO water	3.60	0.3	91.00	3.1	378.20	3.3	4.18	0.2
Control MNB	Tap water	7.63	0.1	105.00	3.0	248.70	2.80	3.14	0.1
	RO water	7.30	0.2	56.00	3.2	257.60	3.1	2.91	0.2
Control Water	Tap water	7.06	0.1	64.00	2.0	322.30	5.65	3.14	0.1
	RO water	7.10	0.2	28.0	3.0	195.40	3.1	3.29	0.2

4.2 Graphical Results

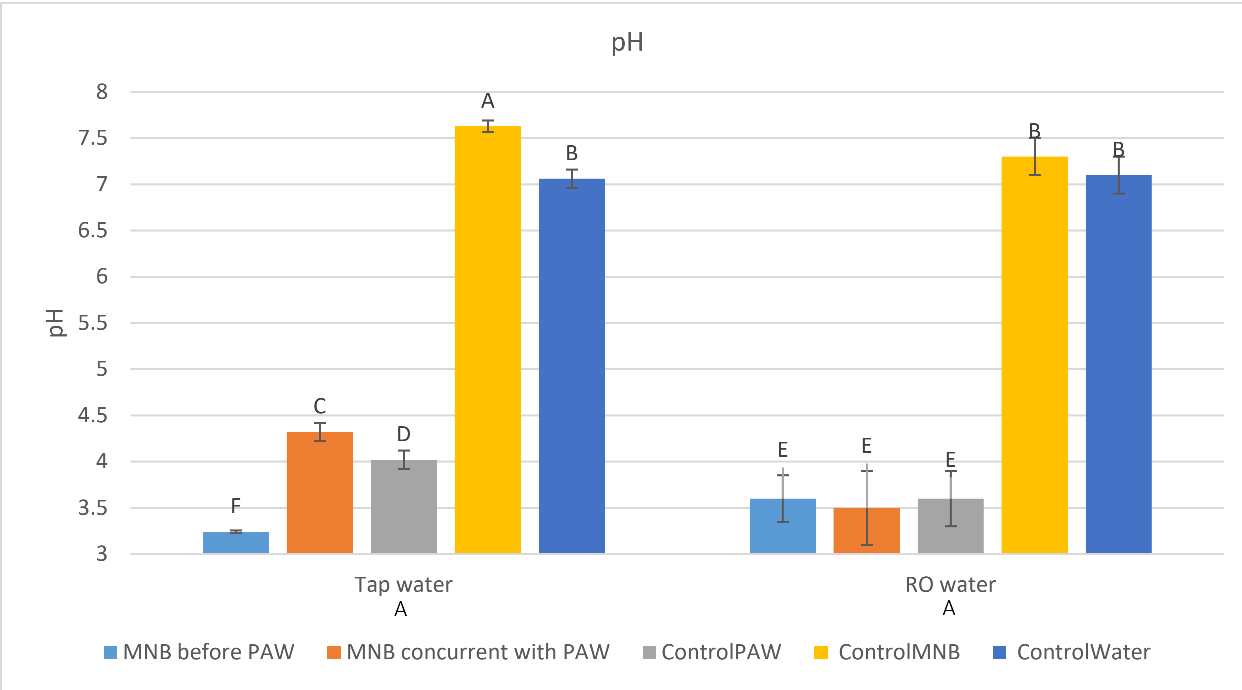

Figure 1. The pH effectiveness of PAW combined with MNB approaches to test in an experiment

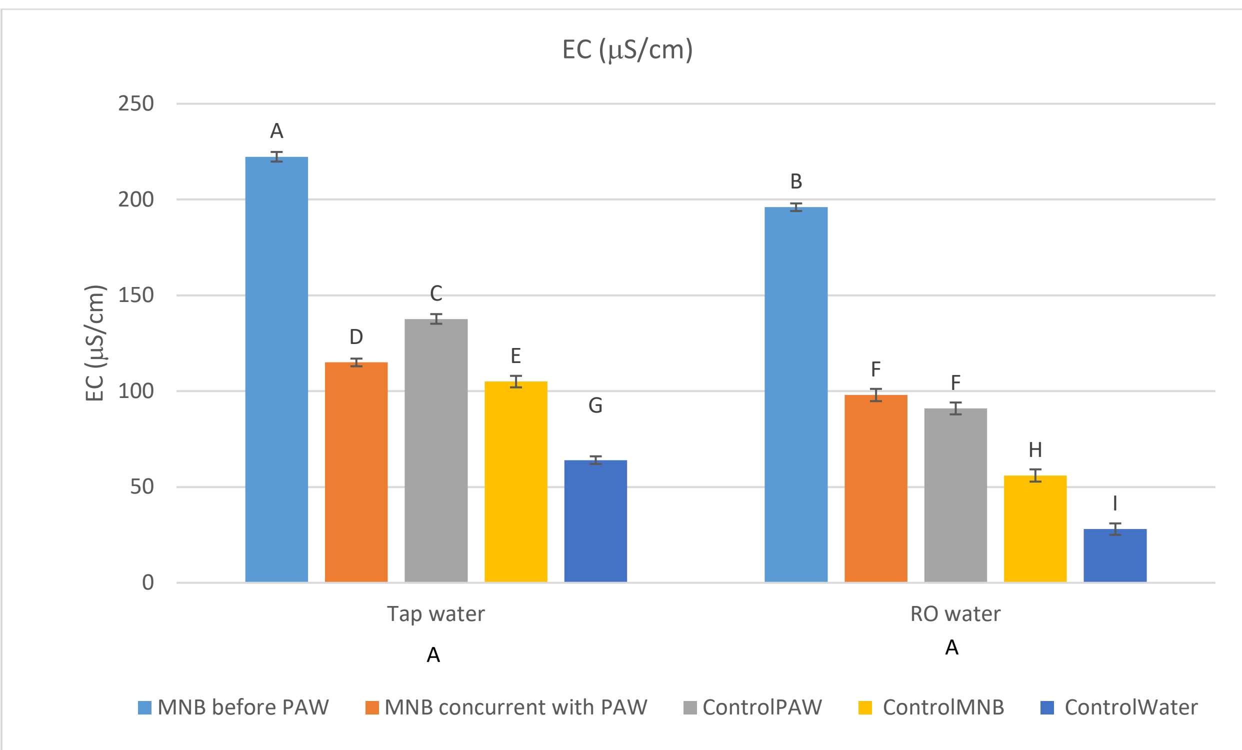

Figure 2. The EC effectiveness of PAW combined with MNB approaches to test in an experiment

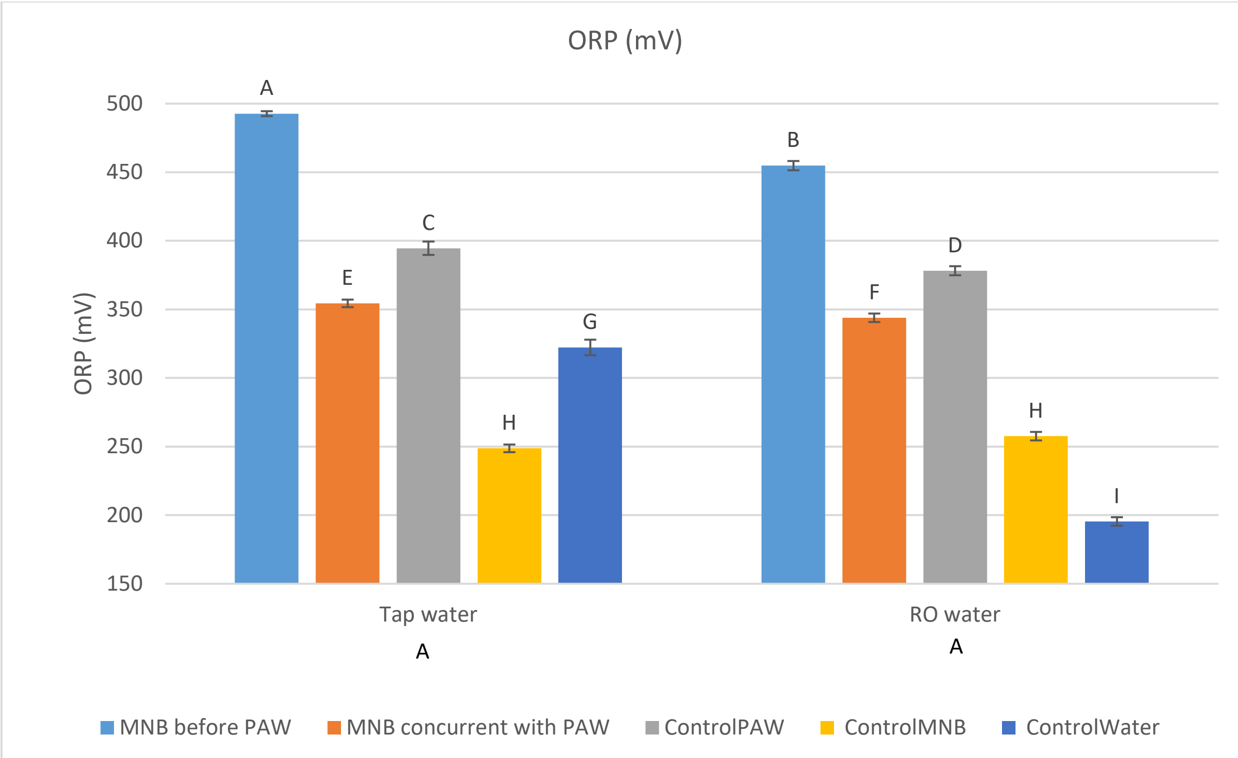

Figure 3. The ORP effectiveness of PAW combined with MNB approaches to test in an experiment

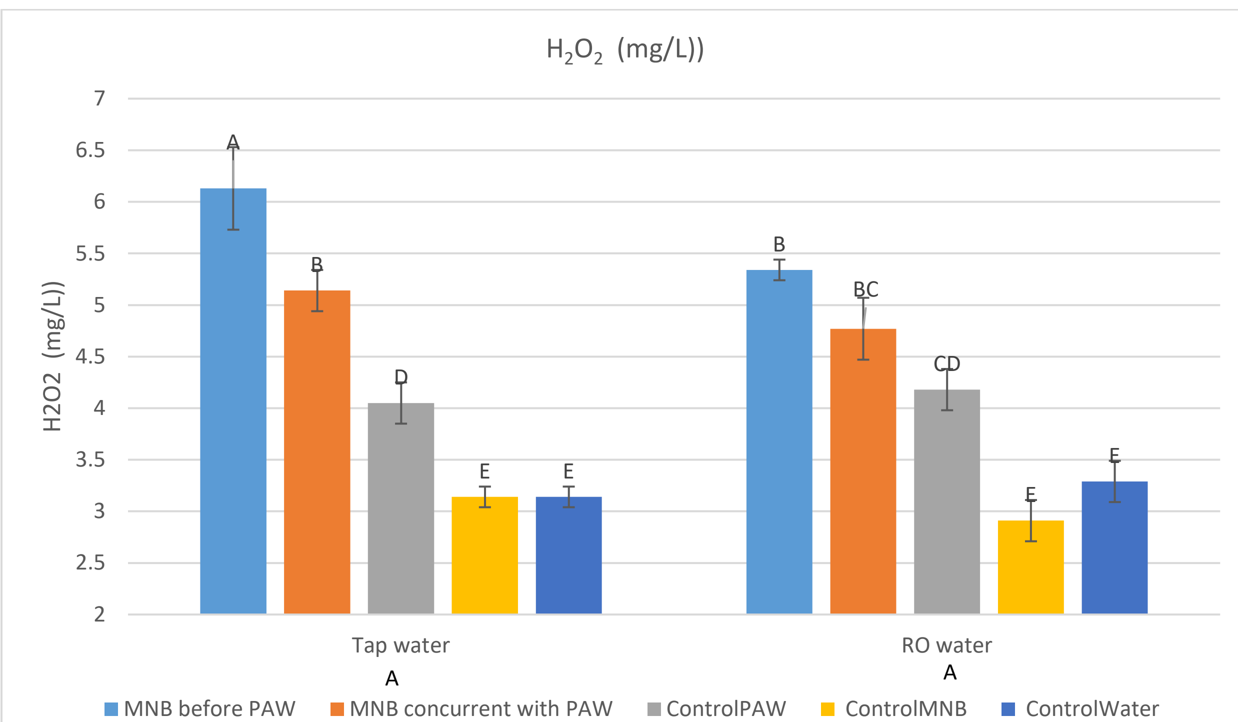

Figure 4. The H_2O_2 effectiveness of PAW combined with MNB approaches to test in an experiment

6. Conclusion

This research investigated the enhancement of PAW by combining with MNB to increase the efficiency of disinfection using EC pH ORP and H_2O_2 measurements as chemical properties evaluation. The factors under investigation include the sequence of MNB and PAW and the type of water. The sequence experiment significantly affects EC, ORP, and H_2O_2 concentration, but not pH. Therefore, MNB should be used initially before PAW. Furthermore, the water type also significantly affects EC ORP and H_2O_2 concentration. In this study, tap water usually has a higher content of minerals than RO water, showing a more significant value of chemical properties after being treated with MNB and PAW. Therefore, tap water in the MNB/PAW experiment could have higher efficacy than RO water. Since the main effect of disinfection came from ROS, H_2O_2 concentration is the key to representing the efficacy of this combination technique. Consequently, the appropriate conditions for this study would be the treatment of tap water with MNB first and followed by PAW could lead to the highest amount of H_2O_2 concentration, ORP, and EC, which are 6.13 mg/L 492.7, and 222.33 μS/cm, respectively. Further research is needed to determine the impact of PAW combined with MNB technology on foodborne pathogens, and the optimal conditions for pathogen inactivate in the poultry industry.

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