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The role of ionized water as a safe alternative to disinfectants in poultry Slaughter houses

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ABSTRACT

Recently, electrolyzed water a sustainable and eco-friendly disinfectant made by electrolyzing an acid or salt has gained a lot of popularity due to its usage in a variety of industries, including food, medicine and agriculture. Electrolyzed water (SALEW, pH 8–10) with a slightly alkaline pH has gained popularity as a substitute disinfectant for cleaning chicken houses. Some aspects of EW, such as the many processes for SALEW formation and the antibacterial action of SALEW, are still poorly understood. Therefore, the goal of the current study was to assess the advantages of using freshly made SALEW in place of chlorine on 75 samples of chicken breast fillets. The samples were tested for *Salmonella typhimurium* and *Listeria monocytogenes* count, before and after treatment with SALEW in comparing with chlorine 50 ppm The results were promising and the effect of SALEW showed decreasing in the *Salmonella typhimurium* and *Listeria monocytogenes* count. The concentration of these bacteria dramatically decreased after treatment, with Chlorine 50 ppm and slightly alkaline electrolytes water. The antibacterial effect of the SALEW was decrease gradually by time. The 2nd day was the best of the antibacterial effect of the SALEW. Chlorine shows higher disinfectant effect against *Listeria monocytogene* than SALEW at day 2, 3 and 4 of treatment. SALEW is an effective disinfectant, with several advantages such as cheap, environmentally friendly, and safe production

INTRODUCTION

Growth in the population raises the total demand for food, but changes in wealth have an impact on consumption habits (**Miladinov, 2023**). The increasing demand is concerning as the large-scale animal husbandry practices now in use have been connected to issues with public health, environmental damage, and animal suffering. The animal agricultural sector is linked to foodborne sickness, diet-related dis-

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eases, antibiotic resistance, and infectious diseases in terms of human health (**Wolk, 2017 and Rubio et al. 2020**). Meat products are highly vulnerable to contamination from food-borne pathogens, such as *Salmonella* (**Morton et al. 2019**), *Listeria monocytogenes* (**Cabal et al. 2019 and Tchatchouang et al. 2020**), and other pathogens associated with slaughterhouses, processing facilities, and international outbreaks (**Ramírez Orejel and Cano-Buendía, 2020**). One of the main components of an effective HACCP program in the food business is procedures that attempt to reduce or even eliminate pathogens from surfaces. These procedures also regulate food contamination in households, food markets, restaurants, medical facilities, and public areas (**Tomasello et al. 2021**).

The microbiological safety of chicken meat has been enhanced by various methods for lowering bacterial contaminations (**Ishaq et al. 2020**). To improve the safety and quality of fresh poultry meat products before refrigeration, several sanitizing procedures have recently been put into place (**Sheng et al. 2018**). Additionally, because chemicals may be hazardous to human health, consumers are worried about the use of chemicals as a sanitizing agent. Because of this, the majority of research on decontaminating fresh chicken meat or vegetables has concentrated on sanitizing agents other than chemical sanitizers, such as hydrogen peroxide, peracetic acid, and chlorine (**Petri et al. 2021**). Furthermore, several organisms, including Salmonella, are developing resistance to these conventional disinfectants. Free chlorine also influences chemical sanitizer effectiveness, the amount of organic material present, the number of microorganisms, the quality of the washing water, etc. (**Roobab et al. 2023**).

As an antimicrobial treatment technology that has garnered attention recently because of its verified uses in the food industry, electrolyzed water (EW) is one of the green cleaners on the market. It has been utilized as a disinfectant and sanitizing agent in the food industry. Additionally, it has been shown that the suspensions' EW has a more substantial effect on food and equipment surfaces (**Attia et al. 2021**). Research is being done to determine if acidic, neutral, or alkaline electrolyzed water is effective against microorganisms in biological systems. Cleaning and other uses for alkaline electrolyzed fluids, such as antibacterial applications, are successful (**Iram et al. 2021**). Sodium hydroxide, which dissolves grease, is a component of alkaline electrolyzed water (ALEW), created at the cathode. Fouling deposit will expand when it comes into contact with NaOH, helping to remove dirt (**Khalid et al. 2016**). At 4 °C and 25 °C, the antibacterial activity of acidic electrolyzed water was assessed against *Salmonella typhimurium* and *Listeria monocytogenes*. The greatest log decrease of almost 8 CFU/mL at 25 °C was demonstrated by the data (**Rebezov et al. 2022**).

Studies show that the controversial approach used by poultry manufacturing businesses to wash chickens after they are slaughtered with chlorine fails to eliminate all harmful bacterial contamination. While cooking might mitigate this issue, chicken that has been chlorine-washed can contaminate a kitchen, and several European countries prohibit the importation of chicken that has been chlorine-washed due to animal welfare concerns. This study aimed to demonstrate the impact of five minutes of immersion in slightly alkaline electrolyzed water on the counts of *Salmonella typhimurium* and *Listeria monocytogenes* in the samples under investigation.

MATERIALS and METHODS

Sample collection and preparation

A total of 75 samples of chicken breast fillets are assembled from a poultry massacre. They were collected just after slaughtering and washing only with tap water without any additives and were transferred to the lab in an ice box as soon as possible. They are divided into 5 groups, each set containing (15 samples); 1st group washed with regular water (control), 2nd group inoculated with salmonella and washed with chlorine by 50 ppm, 3rd group inoculated with listeria and washed with chlorine by 50 ppm,4th group inoculated with salmonella and washed with electrolyzed water, and 5th group inoculated with listeria and washed with ionized water.

Bacterial inoculum preparation

Freeze-dried pure cultures of *Salmonella typhimurium* ATCC 14028 and *Listeria monocytogenes* 10403s were obtained from the Laboratory for Veterinary Quality Control on poultry production. These cultures were resuspended by adding 10 mL sterile tryptic soy broth (TSB; MB Cell) and cultured at 37°C for 24 h (**Kim et al. 2019**).

Inoculation of the samples

The skinless chicken breasts (approximately 170–200 g) were obtained immediately after slaughter from a local slaughterhouse and inoculated with each bacteria (*S. Typhimurium* and *L. monocytogenes*) by dipping in a 700 mL solution containing 70 mL TSB at the level of 10^8 and 630 mL sterile saline solution for 15 min at 23°C according to the method of **Alonso-Hernando et al. (2015)**. Then, the chicken breasts were dried for 20 min at 23°C in a clean room.

Slightly alkaline electrolyzed water (SALEW) preparation according to **Athayde et al. (2018)** and **Tolba et al. (2023)**

By electrolyzing tap water with sodium chloride (NaCl) 0.2% (2 g for every litre of tap water), electrolyzed water (EW) of SAlEW (pH, 8.5) was produced. For 10 minutes, an electrolysis chamber with two poles—the cathode (-) and anode (+) was subjected to a current of 9–10 volts and 8–10 amber. Ions were exchanged over a bridge between two different sides. Sodium hydroxide (NaOH) production on the cathode side led to the formation of SAlEW. A digital meter was used to assess the pH of EW (**FSSAI, 2015**).

Microbiological analysis

About 25 grams of meat samples were weighed and put into a sterile homogenizer flask that contained 225 milliliters of peptone water (0.1%) under aseptic circumstances. The contents of each flask were homogenized at 14000 rpm for 2.5 minutes to yield a 10^{-1} dilution. After that, a sterile pipette was transferred 1 ml to a sterile test tube containing 9 ml of (0.1%) peptone water. Subsequently, a decimal serial dilution was made in increments of 10^{-10} to accommodate the entire range of anticipated sample contamination (**APHA, 1992**). After SAlEW treatment (day 1), all samples were aseptically and immediately placed in a stomacher bag containing 90 mL of sterile PBS and homogenized for 2 min. After homogenization, 0.1 mL aliquots of the samples were serially diluted in 0.9 mL of sterile PBS as needed, and 0.1 mL of the appropriate dilutions were spread-plated onto each selective medium. All inoculated agar plates were incubated at 37°C for 1–2 days, following which the CFU levels were enumerated. Then the same steps were carried out at 2nd, 3rd and 4th day.

pH measurement (ES 63-11/2006)

We conducted a confirmation using a Digital Jenco 609 pH meter. The pH was measured by combining a 10 g sample with 90 ml deionized water for two minutes.

Sensory evaluation

Chicken breast fillet sensory evaluation was performed on days one till day 4. Every sample was assessed three times. Following the guideline table, a straightforward four-point scoring system was used to determine colour, odour, and texture. The following formula was used to determine the sensory index (**Moghassem Hamidi et al., 2021).**

SI = (2X C) + (2X O) +T

5C stands for colour, O for odour, T for texture, and SI for sensory index. It assesses chicken breast flesh's colour, texture, and odour to determine its sensory quality score. Qualities: 4 (Highest quality), 3 (Good quality), 2 (Fair quality), 1 (Poor quality).

Statistical analysis

Each treatment's mean and standard deviations were computed using data collected in independent replication experiments. The general linear model approach of the SPSS 20.0 program (SPSS Inc., Chicago, IL) was used for all the data. The multiple range test by Duncan was utilized to differentiate means, with a significance threshold of P < 0.05.

RESULTS

Sensory evaluation

Table 1 shows the sensory analysis results conducted on the treated chicken breast meat samples from days 1 to 4 of storage. Each treatment's sensory indices of texture, colour, and odour decreased over storage.

Table 1. The mean scores for the sensory attributes of samples of chicken breast fillets stored at 4±1ºC for 4 days while being treated with Chlorine & slightly alkaline water (SAlEW) .

Time (days)/ groups	Control without treatment	Chlorine (50ppm)	Electrolytes water (slightly alkaline water)
Odor			
Day 1	3.6±0.08	3.7±0.08	3.8±0.05
Day 2	3.5±0.08	3.6±0.08	3.7±0.08
Day 3	3.1±0.08	3.2±0.2	3.5±0.08
Day 4	2.5±0.2	2.8±0.05	3.0±0.1
Colour			
Day 1	3.8±0.08	3.8±0.05	3.9±0.05
Day 2	3.4±0.2	3.6±0.08	3.7±0.1
Day 3	2.5±0.2	3±0.2	3.3±0.1
Day 4	1.9±0.1	2.9±0.08	3.0±0.08
Texture			
Day 1	3.7±0.08	3.8±0.08	3.9±0.05
Day 2	3.4±0.08	3.5±0.3	3.5±0.2
Day 3	2.7±0.3	3.2±0.1	3.3±0.1
Day 4	2.0±0.1	2.8±0.08	3.0±0.1

Data is given as mean ± SE of 3 replicates.
Values with different letters within the same row differed significantly at (P<0.05).

Hydrogen ion concentration (pH)

The findings in **Table (2)** demonstrated the variations in the chicken breast flesh samples; the control group's starting pH on day 1 was 5.5±0.08. The last group treated with SAlEW had the lowest pH value of 5.7 ± 0.05 at the end of storage (day 4), whereas the control group had the highest pH value of 6.1 ± 0.08.

Table 2. Pattern of pH of chicken breast fillet samples stored at 4±1ºC while being treated with Chlorine & slightly alkaline water (SAlEW)

Groups/ Time (days)	pH values ±SE			
	Day 1	Day 2	Day 3	Day 4
Control without treatment	5.5±0.08	5.8±0.08	6.0±0.1	6.1±0.08
Chlorine (50ppm)	5.4±0.08	5.6±0.2	5.7±0.08	5.9±0.08
Electrolytes water (slightly alkaline water)	5.3±0.1	5.5±0.05	5.6±0.05	5.7±0.05

Data is given as mean ± SE of 3 replicates.
Values with different letters within the same row differed significantly at ($P<0.05$).

Effect of different disinfection treatments on *Salmonella typhimurium*

As shown in **Table 3 and Figure 1**, the initial (day 1) culturable *Salmonella typhimurium* concentration range without treatment was $79x10^4$. The concentration dramatically decreased after treatment, with Chlorine 50ppm ($66x10^3$) and slightly alkaline electrolytes water ($52x10^3$). Notably, the antibacterial effect of the water of the slightly alkaline electrolyte was decrease gradually by time to be $19X10^5$ at the day 4th. The 2nd day was the best of the antibacterial effect of the slightly alkaline electrolytes water $27X10^3$ The deterioration of the control samples on the third day, the artificial contaminated with microbes, and the control without inoculation of microbes were unacceptable on the fourth day; the samples used only disinfectant without inoculation, and the decomposition began on the fifth day.

Table 3. Effect of different disinfection treatments on *Salmonella typhimurium* in poultry slaughter houses.

Time (days)/ groups	Control without treatment	Samples with Treatments	
		Chlorine (50 ppm)	Electrolytes water (slightly alkaline water)
1st day	$79x10^4$	$66x10^3$	$52x10^3$
2nd day	$46x10^4$	$37x10^3$	$27X10^3$
3rd day	$93X10^5$	$85X10^4$	$22X10^4$
4Th day	$98X10^6$	$42X10^5$	$19X10^5$

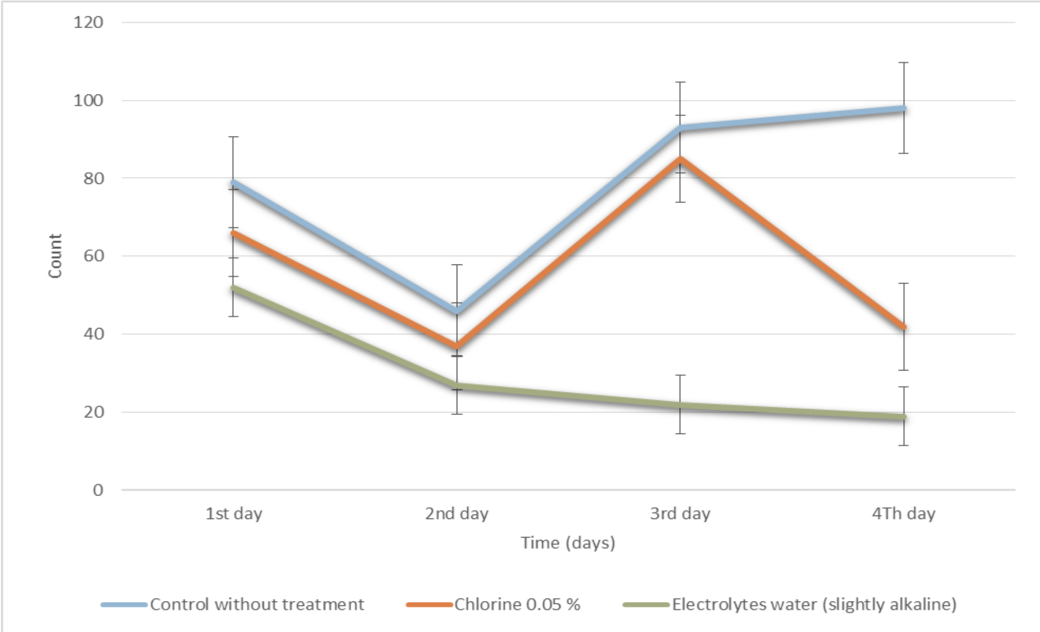

Figure 1. *Salmonella typhimurium* in poultry slaughter houses treated with different disinfection

Effect of different disinfection treatments on *Listeria monocytogene*

As shown in **Table 4 and Figure 2**, the range of the initial (day 1) culturable *Listeria monocytogene* concentration without treatment was $76x10^4$. The concentration then dramatically decreased after treatment, with Chlorine 50ppm ($66x10^3$) and slightly alkaline electrolytes water ($52x10^3$). Notably, the antibacterial effect of the slightly alkaline electrolytes water was decrease gradually by time.

Table 4. Effect of different disinfection treatments on *Listeria monocytogene* in poultry slaughter houses

Time (days)/ groups	Control without treatment	Samples with Treatments	
		Chlorine (50 ppm)	Electrolytes water (slightly alkaline)
1st day	$76x10^4$	$52x10^3$	$18x10^3$
2nd day	$49x10^4$	$42x10^3$	$55x10^3$
3rd day	$95x10^5$	$35x10^4$	$40x10^4$
4Th day	$102x10^6$	$36x10^5$	$49x10^5$

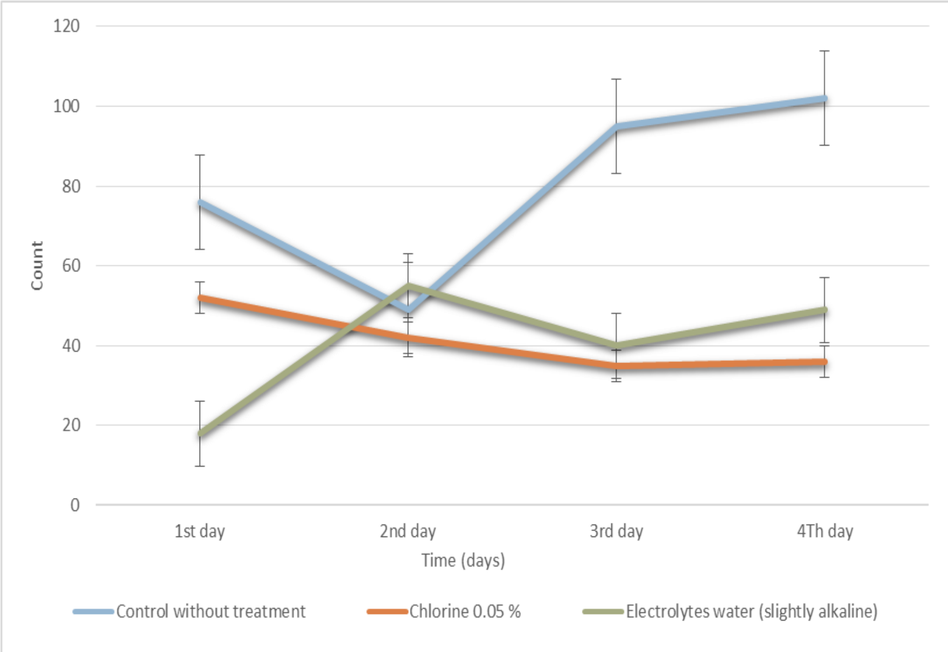

Figure 2. Treatments of *Listeria monocytogene* in poultry slaughter houses with different disinfectants.

DISCUSSION

The results of the sensory analysis conducted on the treated chicken breast meat samples from days 1 to 4 of storage are displayed in **Table (1)**, which demonstrates what the panellists found to be the case for both the treated and untreated samples of freshly prepared chicken breast samples (day 1) with slightly alkaline electrolyzed water (SALEW) in terms of all sensory evaluation. All treatments' sensory indices for texture, colour, and odour decreased after storage (day 4). The chicken breast samples' sensory quality significantly decreased, and they were no longer suitable for cooking after four days of storage, especially in the control sample. Sensory attribute changes were less noticeable when comparing the chicken breasts treated with SAlEW samples to the control samples and other treatment groups.

Hernandez-Pimentel et al. (2020) report that during the refrigerator storage period until the conclusion of the fourth day, the pH values of the chicken breast flesh samples (treated or control groups) increased due to lipid/protein decomposition caused by bacteria, chemicals, and physical damage. The findings in **Table (2)** demonstrated the variations in the chicken breast flesh samples; the control group's starting pH on day 1 was 5.5±0.08. The last group treated with SAlEW had the lowest pH value of 5.7±0.05 at the conclusion of storage (day 4), whereas the control group had the highest pH value of 6.1±0.08. Rising pH readings might be caused by the activity of microbial or endogenous enzymes such as lipase and protease, which increase the concentration of volatile bases after prolonged storage (**Hernández Pimentel et al. 2020**). Additionally, on day four, there was a statistically significant difference between the treatment and control groups. The pH value was higher in the control group compared to the other treatment groups.

Salmonella is bacteria that is responsible for several foodborne outbreaks worldwide, which poses a significant threat to public health. Salmonella strains that have the capacity to form biofilms have been regularly identified from a variety of food processing facilities, particularly in the poultry sector (**Pang et al. 2023**). Furthermore, Listeria species are crucial to the food sector since they can cause meningitis, miscarriages, and even death. Food and the environment, particularly the food industry, are sources of *L. monocytogenes* (**Kara and Aslan 2021 & Sepin and Pamuk 2021**). Despite several attempts, it is still difficult to totally eradicate illnesses like Salmonella and Listeria (**Bodie et al. 2023 and Teklemariam et al. 2023**).

Due to their strong bactericidal properties, the components of acid and peroxymonosulfate are frequently utilized as chemical agents for disinfection and meet all food, animal, and public health regulations (**Bai et al. 2022**). In the chicken supply chain, chlorine disinfectants are frequently employed; nevertheless, this exposure can also lead to the development of bacterial tolerance to chlorine, which is frequently connected to antibiotic cross-resistance (**Xiao et al. 2022**). Due to its affordability, ease of use, and environmental friendliness, electrolyzed water has emerged as a reliable substitute for sanitization in the food business. According to **Yan et al. (2021) and Chen et al. (2022)**, most investigations have focused on using electrolyzed water to show the antibacterial activity of meat and meat products.

This study, we proved that a special electrolyzed water SALEW was an efficient and safe disinfectant against Gram-negative bacteria. Thus far, research on the antibacterial properties of acidic electrolyzed water has mostly concentrated on the impact of free available chlorine (FAC) in the electrolyzed water. Neutral electrolyzed water (pH 7) has been shown to be either as effective as or more efficient than other popular chlorine-based sanitizers in reducing pathogens (**Ogunniyi et al. 2019**).

Because of its strong disinfection activity and ease of use, alkaline electrolyzed water (ALEW) is a good substitute for many other more popular disinfectants (**Tomasello et al. 2021**).

The current study investigated the potential of SALEW as a disinfectant against Listeria and Salmonella isolates from slaughtered chicken houses. Several investigators studied how EW affected various food types by inactivating and eliminating *L. monocytogenes* and *Salmonella Spp.* Spraying chicken carcasses with EW reduced Salmonella by 2.7 log10 (**Northcutt et al. 2007**). Under conditions similar to those of an industrial processing facility, **Kim et al. (2005)** examined the effectiveness of EW in preventing and eliminating fecal pollutants on chicken carcasses. In this study, the mean value of *Salmonella typhimurium* count at the first day decreased from $79x10^4$ before the treatment, with chlorine 50ppm treatment it was $66x10^3$ and $52x10^3$ after using slightly alkaline electrolyzed water, which proves the disinfectant effect of the SALEW. In the 2nd day, *Salmonella typhimurium* count decreased from $46x10^4$ before the treatment, with chlorine 50ppm treatment it was $37x10^3$ and $27x10^3$ after treatment with SALEW. SALEW shows its effect on *Salmonella typhimurium* due to high oxidation reduction potential and pH. But these properties also make SALEW non-stable and susceptible against organic materials. Our study achieved the most significant reduction with SALEW treatment and the control. Our findings supported that SALEW is unstable and losing its efficacy on days 3 and 4.

In this study, the mean value of *Listeria monocytogene* count at the first day decreased from $76x10^4$ before the treatment, with chlorine 50ppm treatment it was 52×10^3 and 18×10^3 after using slightly alkaline electrolyzed water, which proves the disinfectant effect of the SALEW. In the 2nd day, *Listeria monocytogene* count decreased from $49x10^4$ before the treatment, with chlorine 50ppm treatment it was 42×10^3 and 55×10^3 after treatment with SALEW. According to **Ovissipour et al. (2018)**, even at different temperatures, the inactivation of *L. monocytogenes* in salmon fillets was more successful when done with acidic and neutral electrolyzed water. At days 2, 3, and 4 of treatment, chlorine had a stronger disinfection effect than SALEW against *L. monocytogene*. In addition to sanitizer concentration, contact duration, and treatment techniques, the presence of organic matter, pH, temperature, and produce physiology all significantly affect how well a chlorine solution decontaminates (**Jyoti Aryal et al. 2024**). Our investigation did not find any significant differences ($P < 0.05$) between the two contact strategies from each bacterium.

CONCLUSION

It is feasible to conclude that SAlEW can delay chemical and microbiological modifications, extend the shelf life of chicken meat, and enhance the overall odour, texture, and colour of chicken breast meat. Treatments with SAlEW considerably decreased the number of aerobic bacteria in chicken breast meat samples compared to control and other treated groups. This is believed to enhance microbiological quality, increase shelf life, and support oxidation stability of the meat samples while stored at 4°C. SAlEW significantly lowers the microbial load of S. *Typhimurium* and *L. monocytogenes* in the chicken breast flesh samples. Therefore, SAlEW provides a practical, safe, and effective way to eliminate germs, a significant public health risk. Though further research is needed to confirm SAlEW's usefulness before it may be employed in the food sector, the study's optimistic findings suggest that it might be a viable alternative for preserving chicken meat without affecting its sensory attributes. According to disinfectants ' guidelines, poultry meat had a five-day shelf life at 4 °C in freezers.

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