



Oxidized Water Vs. Other Disinfectants: A Comparative Study on the Risks Control of Food contact surfaces Contaminated with *Escherichia coli* and *Salmonella*



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Abstract

SEVERAL treatments have been demonstrated to be effective in disinfecting laboratory-contaminated stainless-steel surfaces inoculated with reference strains of *Salmonella* and *E. coli*. These treatments included sodium hypochlorite (100 & 150 ppm), hydrogen peroxide (1% & 3%) and acidic electrolyzed water (AEW) at pH 2.5-3 and slightly acidic electrolyzed water (SAEW) at pH 5-6.5. The best efficiency was demonstrated by AEW at pH 2.5-3, which completely eliminated *S. typhimurium* and *E. coli*, which had initially bacterial loads of 5.83 log CFU for *E. coli* and 5.7 log CFU for *Salmonella*. Additionally, *E. coli* contamination was completely eliminated using sodium hypochlorite at 150 ppm. The reduction in bacterial load was evaluated, and reduction scales along with risk factors (RF) were calculated for both *E. coli* and *S. typhimurium*. The recorded reduction scales were: 0.2, 1.4, 0.2, 0.35, 0.0, and 0.06 by using NaClO (100 & 150 ppm), H₂O₂ (1% & 3%), Acidic Electrolyzed Water (AEW) with a pH range of 2.5-3 & 5-6.5, respectively. Risk assessments at 100 ppm of NaClO yielded high risk levels (5.7 and 6) for both organisms. Application of 150 ppm NaClO resulted in negligible risk for *E. coli* (0) but a high risk (3.8) for *S. typhimurium*. Conversely, 1% H₂O₂ produced very high-risk levels (6.3 and 6.2) for both organisms. Using 3% H₂O₂, risk levels were high (3.2) for *E. coli* and moderate (3) for *S. typhimurium*. Acidic Electrolyzed Water demonstrated negligible risk for both organisms, while Slightly Acidic Electrolyzed Water resulted in moderate risk levels (2.9) for both this risk.

Keywords: Electrolyzed water (AEW & SAEW), Sodium hypochlorite (NaClO), Hydrogen peroxide.

Introduction

Escherichia coli categorized as a facultative anaerobe with a rod-like form that is Gram-negative bacteria. Both humans and warm-blooded animals naturally contain it in their intestinal flora, and it is a reliable indicator of fecal contamination. While some *E. coli* strains are harmless, others are pathogenic to humans. Pathogenic strains can cause food poisoning, as well as infections of the urinary tract, lungs (pneumonia), blood (bacteremia), and intestines. Five main types of *E. coli* cause intestinal infections: enter-toxigenic (ETEC), enter aggregative (EAggEC), enter pathogenic (EPEC), Enter invasive (EIEC), and enterohemorrhagic (EHEC) or verotoxin producers (VTEC) [1]. The presence of *Escherichia coli* in ready-to-eat foods points to poor hygienic practices, implying either contamination or insufficient thermal treatment. Ideally, these products

should be entirely free of *E. coli*. A level below 20 CFU/g is deemed the acceptable quality standard for this microorganism. In fish and other food products, concentrations ranging from 20 to 100 CFU/g classified as borderline or intermediate, whereas levels above 100 CFU/g considered unacceptable and signify substantial contamination [2].

Salmonella enterica is a rod-shaped, gram-negative, non-sporulating aerobic or facultatively anaerobic bacterium that is a member of the *Enterobacteriaceae* family. There are more than 2,600 known *S. enterica* serovars, and they can be found in both clinically healthy and sick animals as well as food and its byproducts. "Salmonella" is used to identify serovars, which are then followed by the serovar name, both capitalized and non-italicized (e.g., *S. typhimurium*) [3]. *Salmonella spp.* infections of poultry and poultry products, as well as contact

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surfaces, provide an urgent risk to public health since they can result in food poisoning and, in cases of severe infection, may cause death in immunocompromised people [4]. Outbreaks of salmonellosis, a common food poisoning symptom caused by *Salmonella spp.*, are regularly reported worldwide and include symptoms like vomiting, fever, and stomach pain. The worldwide *Salmonella* surveillance and laboratory assistance initiative was launched in 2002 [5].

Microbial contamination represents a serious risk to food safety. The presence of harmful microorganisms in food products, on contact surfaces, or in raw materials can cause foodborne diseases and outbreaks. *Salmonella enterica* continues to be a leading bacterial pathogen affecting public health. Ingesting food contaminated with *Salmonella* or meeting contaminated surfaces can lead to food poisoning, a digestive tract infection that remains a global issue, affecting approximately 3.4 million people each year [6]. *Salmonella* contamination in food is a major global cause of infection. This contamination can happen through multiple pathways, such as direct contact between food and contaminated surfaces used in food preparation or handling [7]. Cutting boards, knives, and conveyor belts used in food processing are a few examples of these surfaces. Applying disinfectants or active chemical agents to surfaces that come into contact with food is one way to mitigate this risk. It has been demonstrated that using disinfectants can successfully stop the growth of microorganisms and help get rid of foodborne pathogens like *Salmonella*. The rising incidence of pathogenic microorganisms in recent years has underscored the critical need for effective disinfection practices across various fields. Notably, the COVID-19 pandemic has intensified the pursuit of affordable and environmentally sustainable disinfectants. Despite the widespread use of numerous disinfecting agents, they often pose challenges, including the persistence of chemical residues, limited effectiveness, elevated costs, and harmful environmental consequences [8].

Electrolyzed water (EW) has gained recognition as a viable and eco-friendly disinfectant, offering a practical alternative to traditional methods. Its straightforward production process and application, without the need for hazardous chemicals, have led to its increasing adoption. Research has shown its efficacy in various applications, including food sanitization, environmental disinfection [9].

Sodium chloride (NaCl) is electrolyzed in an electrolysis chamber, usually using a membrane or diaphragm to keep the anode and cathode apart, to produce electrolyzed water (EW). Recent studies, however, have developed novel electrolysis generators that do away with the requirement for these diaphragms. In the electrolyzer's single chamber [10], slightly acidic electrolyzed water

(SAEW) with a pH between 5.0 and 6.5 is produced by electrolyzing sodium chloride (NaCl) or hydrochloric acid (HCl). At this pH range, chlorine primarily found as hypochlorous acid (HOCl), a highly effective germicidal agent that exhibits about 80 times the efficacy of an equivalent concentration of hypochlorite ion (ClO⁻) [9]. A number of electrolysis parameters, such as the type and source of water, the type and concentration of electrolyte employed, and the electrical source, influence the properties of electrolyzed water (EW). EW is generally divided into three categories: basic/alkaline electrolyzed water (BEW), neutral electrolyzed water (NEW), and acidic electrolyzed water (AEW). Other variations have developed, including slightly alkaline electrolyzed water (SAIEW), weakly acidic electrolyzed water (WAEW), and slightly acidic electrolyzed water (SAEW) [11].

Hydrogen peroxide (H₂O₂) is a chemical compound that appears as a very pale blue liquid in its pure state, with a slightly thicker consistency than water. It serves as an oxidizer, bleaching agent, and antiseptic. For consumer purposes, it is commonly used in diluted aqueous solutions (3–6% by weight), while higher concentrations are employed in industrial applications. Hydrogen peroxide, the simplest form of peroxide, features a single bond between two oxygen atoms and is classified as a reactive oxygen species. It breaks down gradually into water and oxygen when exposed to light, and much more quickly when it comes into contact with organic or reactive substances. Hydrogen peroxide is usually stored in opaque containers with a stabilizer added to a weakly acidic solution to maintain its stability. It is also naturally present in biological systems, including the human body. Enzymes that either use or degrade hydrogen peroxide are categorized as peroxidases [12].

Hydrogen peroxide (H₂O₂) functions as an antibacterial, immediately killing microbes rather than offering a long-term preserving effect. The production of other strong oxidants, such as singlet oxygen, superoxide radicals, and hydroxyl radicals, is mostly responsible for hydrogen peroxide's antimicrobial activity rather than only its intrinsic oxidative qualities. Enzymes, membrane constituents, DNA, and other biological components are all irreversibly damaged by these reactive oxygen species. The body of a living thing spontaneously produces H₂O₂ to destroy bacteria [13].

Chlorine has broadly utilized as an antimicrobial agent with the objective of alleviating bacterial cross-contamination in poultry carcasses during immersion chilling methods and throughout assorted surfaces in poultry processing environments [14].

The use of disinfectants in processing facilities, especially in chiller tanks, forbidden in the European Union [15]. In Japan, sodium hypochlorite (NaClO)

is utilized at concentrations ranging from 35 to 200 ppm, quantified as total chlorine, for the disinfection of poultry carcasses. Nevertheless, instances of cross-contamination continue to occur, particularly during the chilling phase within slaughterhouses, with pathogenic bacteria still being identified in poultry meat subsequent to processing or at retail markets [16]. Munther et al. [17] indicated that while chlorine management is crucial for diminishing *Escherichia coli* levels during the chilling process, its efficacy in mitigating cross-contamination challenges is comparatively limited. Once bacteria adhere to the surface of chicken meat, their removal or elimination becomes increasingly complex [18]. Consequently, it is deemed preferable to eliminate bacteria prior to their adhesion to poultry meat in order to minimize the risk of cross-contamination.

Thus, this research endeavors to assess the effectiveness of various disinfectants (hydrogen peroxide at concentrations of 1% and 3%) and sanitizers (chlorine at 100 ppm and 150 ppm), as well as acidic electrolyzed water (AEW, pH 2.5-3) and slightly acidic electrolyzed water (SAEW, pH 5.0-6.5), against reference strains of *E. coli* and *S. typhimurium* that have been experimentally contaminated onto food contact surfaces.

Material and Methods

Sample preparation

Seven stainless steel surfaces were experimentally prepared to simulate stainless steel surfaces in food processing plants, inoculated with a reference strain of *Escherichia coli* (ATCC, 25922), and seven additional surfaces were inoculated with *S. typhimurium* (ATCC, 14028), each at a concentration of 10^6 cfu / cm² (totaling 14 food contact surfaces). These surfaces were categorized into four groups:

- Group 1: Control group for the enumeration of the initial load of both organisms.
- Group 2: Disinfection employing chlorine (sodium hypochlorite) at concentrations of 100 ppm and 150 ppm (4 surfaces, 2 for each organism).
- Group 3: Sterilization of four surfaces (2 for each organism) using H₂O₂ (1% and 3%).
- Group 4: Sterilization of four surfaces (2 for each organism) utilizing AEW (pH, 2.5-3) and SAEW (pH, 5-6.5).
- The sterilization time was standardized to 10 minutes for each treated group.
- The experiment was replicated three times to facilitate subsequent statistical analysis.

Preparation of tested strains

According [19] working solutions of *S. typhimurium* and *E. coli* were prepared from

reference stock solutions that had been preserved at -80°C within cryovials. A single bead was resuspended in brain heart infusion broth (Oxoid) and incubated overnight at 37°C for duration of 24 hours prior to the experiments, thereby achieving a final viable count of approximately 10^9 CFU/ml. A serial dilution was performed using physiological saline to yield a concentration of approximately 10^6 CFU/ml, which was employed to contaminate the food contact surfaces, all while conducting the experiment under strictly aseptic conditions.

Enumeration of B-glucuronidase-positive *Escherichia coli* according to (ISO 16649-2:2001) (TBX method)

This methodology serves for the enumeration and isolation of B-glucuronidase-positive *Escherichia coli* in various types of food and feed derived from animal origin, by cultivating the organism on tryptone-bile-glucuronide medium (TBX) at 44°C for a period of 24 hours. Positive plates exhibited blue-green colonies [20].

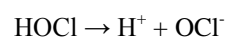
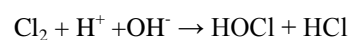
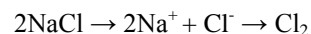
Enumeration and Isolation of *S. typhimurium* according to (ISO 6579-1, 2017). [21].

Preparation of SAEW and AEW according to Tolba et al. [22].

Using an electrolysis device, an electric current (10 A & 9 V) passes through a sodium chloride (NaCl) solution for 30 minutes to create electrolyzed water. The device is composed of an anode and a cathode, both of which are composed of iron metal.

Slightly Acidic Electrolyzed Water (SAEW)

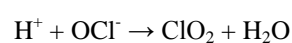
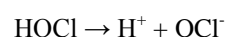
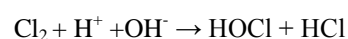
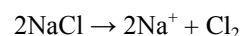
It is generated with a moderate electric current and a low concentration of NaCl (typically 0.1%).



The resulting SAEW has a pH range of 5.0-6.5 and an oxidation-reduction potential (ORP) of around 700-900 mV. (average 800 mV)

Strong Acidic Electrolyzed Water (AEW)

A higher concentration of NaCl (usually 1.0%) and a higher electric current are used to prepare AEW.



The resulting AEW has a pH range of 2.0-3.5 and an ORP of around 1000-1200 mV. (Average 1100 mV)

The prepared Strongly Acidic Electrolyzed Water (SAEW) and Acidic Electrolyzed Water (AEW) were used either immediately or after a maximum of two days of storage in separate containers at 4°C.

Risk Assessment and Risk Factor Calculation according to ISO 45001:2018 [23].and Eric Graves [24]

In the context of risk assessment, the risk factor (RF) is calculated based on available data regarding the probability of contamination and the consequence (impact) of the pathogen. The probability is often estimated based on the contamination level, categorized as follows:

-Low contamination level: $< 1 \log \text{ CFU/g}$, corresponding to a low probability (0-0.3)

Moderate contamination level: $1-3 \log \text{ CFU/g}$, corresponding to a medium probability (0.4-0.6)

-High contamination level: $3-5 \log \text{ CFU/g}$, corresponding to a high probability (0.7-0.9)

-Very high contamination level: $> 5 \log \text{ CFU/g}$, corresponding to a very high probability (1)

While the consequence category, is classified as follows:

- 1.Negligible (1): No significant harm or impact.
- 2.Low (2-3): Minor harm or impact, easily recoverable.
- 3.Moderate (4-5): Significant harm or impact, some recovery possible.
- 4.High (6-7): Major harm or impact, difficult recovery.
- 5.Very High (8-9): Extreme harm or impact, long-term or irreversible consequences.
- 6.Catastrophic (10): Severe, widespread, and irreversible harm or impact.

The risk factor (RF) is calculated by multiplying the probability x consequence categories:

RF = Probability x Consequence Risk Level Categorization

Based on the calculated RF values, the risk can be categorized into different levels

1. Very Low Risk ($0.0 - \leq 0.1$): No significant risk or impact.
2. Low Risk ($>0.1 - \leq 1$): Minor risk or impact, easily manageable.
3. Moderate Risk ($>1 - \leq 3$): Significant risk or impact requires attention and mitigation.
4. High Risk ($>3 - \leq 6$): Major risk or impact, requires immediate attention and mitigation.

5. Very High Risk (>6): Extreme risk or impact requires urgent attention and mitigation.

These risk levels facilitate decision-making and enable the implementation of appropriate measures to mitigate or manage the risks associated with *E. coli* and *Salmonella* contamination.

Statistical analysis

Statistical Packaging for the Social Sciences (SPSS) Ver. 20 was used to perform a triplicate statistical analysis of the collected data, and the mean and standard deviation (Mean \pm SD) were the outcomes. Analysis of variance was used to examine the data (one-way ANOVA). The results were deemed statistically significant if the p-value was less than 0.05 ($p \leq 0.05$).

List of preservatives versus the concentration of each disinfectant and sanitizers used in the experiment were listed in Table (A)

Results

Table and Figure (1) represents the mean $\log_{10} \text{ CFU/cm}^2 \pm$ standard deviation as well as the log reduction scale (Fig. 2) for *E. coli* and *S. typhimurium* treated with different disinfectants. Represented by sodium hypochlorite (NaClO) and hydrogen peroxide (H_2O_2) alongside electrolyzed water (EW) as a sanitizer. For *E. coli*, mean counts of 4.10 ± 0.10 and $<1 \pm 0.00$ were observed with 100 ppm and 150 ppm NaClO, respectively. Treatment with 1% and 3% H_2O_2 yielded counts of 5.0 ± 0.10 and 1.67 ± 0.15 , respectively. With EW at pH 2.5–3.0 and 5.0–6.5, counts of $<1 \pm 0.0$ and 1.23 ± 0.21 were recorded, respectively, compared to a control count of 5.83 ± 0.06 .

For *S. typhimurium*, mean counts of 4.30 ± 0.20 and 1.40 ± 0.26 were observed with 100 ppm and 150 ppm NaClO, respectively. Treatment with 1% and 3% H_2O_2 resulted in counts of 4.80 ± 0.10 and 1.32 ± 0.03 , respectively. With EW at pH 2.5–3.0 and 5.0–6.5, counts of $<1 \pm 0.00$ and 1.17 ± 0.12 were recorded, respectively, compared to a control count of 5.70 ± 0.10 .

Statistical analysis of *E. coli* counts revealed significant differences ($P < 0.05$) between the control group and all treatment groups, except for the comparison of 150 ppm NaClO and AEW at pH 2.5–3.0. Notably, both 150 ppm NaClO and AEW at pH 2.5–3.0 resulted in complete elimination of *E. coli* contamination. Furthermore, no significant difference ($P > 0.05$) was found between 3% H_2O_2 and AEW at pH 5.0–6.5, suggesting that these two treatments exhibited similar efficacy in reducing *E. coli* contamination on surfaces.

Statistically significant differences ($P < 0.05$) in *S. typhimurium* counts were observed between the control and all treatments, with the exception of the comparison between 150 ppm NaClO and 3% H_2O_2 ,

as well as the comparison between 150 ppm NaClO and AEW at pH 5.0–6.5. No statistically significant difference ($P > 0.05$) was found within these specific treatment pairings. Notably, only AEW at pH 2.5–3.0 completely eliminated *S. typhimurium* from the contaminated food contact surfaces.

Table (1) and Fig (2) showed the log reduction scale between *E. coli* and *S. typhimurium* as it recorded 0.2 & 1.4 using 100 ppm and 150 ppm NaClO; 0.2 log & 0.53 by using 1% & 3% H_2O_2 and 0.0 & 0.6 log reduction scale by using AEW (pH, 2.5–3) & SAEW (pH, 5–6.5), respectively. The log reduction scale between means of both organisms evaluated as follows:

- High difference (≥ 1 log cfu/g): Indicates a substantial difference between *E. coli* and *S. typhimurium* counts as in case of using of NaClO 150 ppm (< 1 log & 1.4 log).
- A Moderate difference (0.5–1 log): Represents a noticeable effect of the treatment on both bacterial types, with a relatively small difference between them. This was not seen in the current study as there were no values lies between (0.5 – 1 log)
- Low difference (0.1 - 0.5 log): Meaning the effect of treatment is almost of low noticeable variation between both types of bacteria as in treatment using 1% H_2O_2 (5 log and 4.8 log).
- Minimal or no difference (< 0.1 log): A difference of less than 0.1 log in bacterial counts indicates a consistent and equally strong effect on both organisms. AEW at pH 2.5–3.0 demonstrated this (0.0 log scale), meaning a 100% log reduction for both *E. coli* and *S. typhimurium*.

Results in Table (2) and Fig. (3) revealed that oxidized water (AEW) of pH (2.5–3) was the best agent for eliminating *E. coli* by 100% (5.83 log reduction) as well as *S. typhimurium* contamination (5.7 log reduction, 100%), followed by NaClO of 150 ppm by 100% for *E. coli* only while for *S. typhimurium*, the reduction rate was 4.3 log (75.44%). SAEW of pH (5.0–6.5) recorded 4.6 log reduction (78.9%) for *E. coli* and 4.53 log reduction (79.47%) for *S. typhimurium*, Hydrogen peroxide 1% recorded 14.24 % & 15.79 % which considered the least reduction rate and %. While, 3% H_2O_2 recorded 47.11 % & 76.84 % for *E. coli* and *S. Typhimurium*, respectively.

Figure (4) illustrates the varying risk levels associated with *E. coli* and *S. typhimurium* contamination on food contact surfaces after treatment with different disinfectants. Notably, Acidic Electrolyzed Water (AEW) with a pH range of 2.5–3 emerged as the most effective treatment, surpassing all other disinfectants used in the study. AEW demonstrated exceptional efficacy in completely eliminating *E. coli* and *S. typhimurium* cells, resulting in a very low risk factor (no

significant risk or impact). The second most effective treatment was 150 ppm sodium hypochlorite (NaClO), which achieved a significant reduction in *E. coli* contamination. However, *S. typhimurium* still posed a high risk level (RF = 3.8) after treatment with 150 ppm of NaClO which tabulated as a high risk or impact requires attention and mitigation. Other notable treatments included 100 ppm NaClO and 1% hydrogen peroxide (H_2O_2), which showed the worst results in the experiment. By using 100 ppm NaClO the risk factor (RF = 5.7) for *E. coli* considered high risk requires immediate attention and mitigation and RF=6.0 for *S. typhimurium* considered Extreme risk or impact requires urgent attention and mitigation. While, 1% H_2O_2 , RF= 6.3 for *E. coli* and 6.2 for *S. typhimurium*. Meanwhile, AEW of pH (5.0 – 6.5) resulted in RF of 2.9 for both organisms which considered moderate risk (Significant risk or impact requires attention and mitigation), This is in comparison to the initial contamination levels of both microorganisms on food contact surfaces, which were assessed at a risk level of 7 (Very high risk).

Discussion

Active chemical disinfectants have been shown to possess the capability to impede microbial proliferation, effectively eliminate a variety of microorganisms, and diminish the populations of foodborne pathogens [25]. Nevertheless, the formulation and classification of the disinfectant can significantly affect its efficacy against particular pathogens. Distinct serotypes of *Salmonella enterica* may display disparate resistance levels to specific disinfectants, and variances in susceptibility may also be observed among *Salmonella*, *E. coli*, and other pathogenic microorganisms. This variability was evident in the current investigation, where the populations of both *E. coli* and *S. typhimurium* fluctuated in response to the concentrations of the disinfectants and sanitizers employed, such as NaOCl (100 ppm and 150 ppm), H_2O_2 (1% and 3%), and electrolyzed water at pH levels of 2.5–3 and 5–6.5.

Sanitizing impact of NaClO

Numerous scholarly inquiries have examined the effects of diverse disinfectants on various foodborne pathogens and materials in contact with food. Byun et al. [26] indicated that chlorine-based disinfectants, particularly sodium hypochlorite (NaClO) and chlorine dioxide, effectively diminished *Salmonella enteritidis* counts on surfaces that contact food. This observation is congruent with the findings obtained for *S. typhimurium* in the current study. Furthermore, Djebbi-Simmons et al. [27] assessed the efficacy of peroxide-based disinfectants, specifically hydrogen peroxide (H_2O_2), alongside chlorine-based disinfectants, namely sodium hypochlorite (NaOCl), against *S. typhimurium* on food contact surfaces. Their findings revealed that hydrogen peroxide

exhibited superior effectiveness compared to sodium hypochlorite in decreasing *S. typhimurium* concentrations. Specifically, these results aligned with those of the current study in which a 3% H_2O_2 solution attained a 4.38 log reduction (76.84%), in contrast to a 1.4 log reduction (24.56%) achieved with 100 ppm NaClO and a 4.3 log reduction (75.44%) with 150 ppm NaClO.

Byun *et al.* [26] demonstrated that a concentration of 4-4.99% NaClO can achieve a 3.77 log reduction of *Salmonella enteritidis* on food contact surfaces, which is regarded as a moderate level of reduction. Moreover, Djebbi-Simmons *et al.* [27] discovered that NaClO (0.88%) resulted in a moderate log reduction (3.35), while Megahed *et al.* [28] concluded that a concentration of 8.25% NaClO resulted in a significant log reduction (6.5 logs). This concentration is markedly higher than the 100 ppm and 150 ppm (0.01% and 0.015%, respectively) utilized in the present study. Our research demonstrated that these lower concentrations of NaClO could reduce *E. coli* by 1.73 log₁₀ (29.67%) and achieve a complete reduction of 5.83 log₁₀ (100%), which are regarded as substantial reduction values. In the case of *Salmonella*, the same NaClO concentrations resulted in reductions of 1.4 log (24.56%) and 4.3 log (75.44%), which are similarly considered substantial reduction values. A combined treatment of 100 ppm chlorine with both 3% lactic acid and 30% ethanol, as reported by Zhang *et al.* [29], resulted in a 2.55 log reduction, which is categorized as a moderate reduction.

The effectiveness of sodium hypochlorite against *S. typhimurium* can be attributed to its capacity to generate compounds that penetrate microbial cell walls. The damage inflicted upon the cell membrane or wall can lead to the inactivation of microorganisms, culminating in a significant log reduction. However, sodium hypochlorite-based disinfectants (NaClO) exhibit certain limitations. Their effectiveness may be compromised in the presence of organic matter, and they have the potential to generate toxic byproducts detrimental to human health. Elevated concentrations of sodium hypochlorite present a health hazard owing to its carcinogenic properties [30]. Consequently, in compliance with the guidance provided by food safety and consumer health organizations, we utilized NaOCl at reduced concentrations (100 ppm and 150 ppm) within the scope of this investigation.

Nathaly *et al.* [31] concluded that the concentrations of NaClO employed (ranging from 0.36 to 6.36 ppm), the duration of exposure (5 min to 38.5 min), and the temperature (ranging from 5 to 38.5 °C) were insufficient to effectively eliminate *S. Enteritidis* ATCC 13076 and *S. Schwarzengrund* from fish tissues entirely. Therefore, increasing the concentration and prolonging the exposure time to NaClO may serve as a viable alternative to improve

Salmonella elimination rates in fish slaughterhouses. This finding reinforces the conclusion of the present research, which demonstrated that the evaluated concentrations of certain disinfectants and sanitizers, while significantly reducing *E. coli* and *Salmonella* counts, did not achieve complete elimination of both organisms. Therefore, a wider range of concentrations should be explored to ascertain the optimal level for complete eradication without compromising product quality and safety.

Byun *et al.* [26] has demonstrated that the efficacy of NaOCl at a concentration of 100 µg/mL is contingent upon the surface type when curing *S. enteritidis*. Notably, a reduction of 4.91 log CFU/cm² was recorded on stainless steel surfaces. These findings imply that chlorine-based disinfectants, such as NaOCl, are effective for application in the poultry industry, particularly on surfaces that come into contact with food, for the management of both planktonic cells and biofilms of *S. Enteritidis*, thereby enhancing food safety. This is aligned with the results the results observed in the current investigation.

Antimicrobial effect of H₂O₂

Hydrogen peroxide (H_2O_2) is a recognized antimicrobial agent characterized by its broad-spectrum efficacy against pathogenic bacteria, fungi, and viruses. It exists as a colorless liquid and is commercially available in aqueous solutions across a diverse range of concentrations. Its potent oxidizing characteristics and capability to induce oxidative damage within microbial cells have contributed to its widespread application within the food industry.

As noted by Nilima and Jeemit [13], hydrogen peroxide's antimicrobial properties arise from its ability to generate reactive oxygen species, including singlet oxygen, superoxide radicals, and hydroxyl radicals. It impedes the growth of *E. coli* by producing highly toxic hydroxyl radicals. *E. coli*, a prevalent and easily accessible pathogen, can be effectively eliminated by hydrogen peroxide. The oxidative stress instigated by hydrogen peroxide results in a reduction of viable *E. coli* counts, which aligns with the results of the present study.

As indicated in a report submitted by the Center for Food Safety and Risk Assessment section in Hong Kong [32], hydrogen peroxide is approved for use in food processing across various nations, including the United States, Canada, Australia, and New Zealand. Its formidable oxidizing properties were instrumental in its selection for this study as an antimicrobial agent against *E. coli* and *S. typhimurium* on experimentally contaminated food contact surfaces, also render it appropriate for various applications. These encompass its utilization as a bleaching and antimicrobial agent in particular foodstuffs, such as dairy and meat products, as well as for the sanitization of surfaces that come into contact with food and food packaging materials.

In mainland China, the classification of hydrogen peroxide is that of a food processing aid. When hydrogen peroxide is employed in food processing, it is imperative that the quantity utilized be meticulously regulated, employing only the minimum amount necessary to fulfill the desired objective.

Sanitizing effect of EW

A significant benefit of the salt-only electrolysis methodology is the resultant electrolysis of water's (EW's) non-toxicity to humans in addition to its environmental compatibility. This renders it suitable for applications within the food industry, surface sanitization, and general cleaning [22-33]. This aligns with the methodology adopted in this investigation, wherein we employ NaCl to facilitate electrolysis. However, a drawback associated with the use of salt as the electrolyte is the potential for corrosion of the generator components resulting from sustained utilization of highly acidic EW.

Acidic electrolyzed water (AEW), produced at the anode, and consists of a range of chemical species, including ClO^- , HOCl , HCl , Cl_2 , and O_2 . It is characterized by a low pH (2–3.5), a high oxidation-reduction potential (ORP) surpassing 1100 mV, and a free chlorine concentration ranging between 10 and 90 ppm. These attributes confer AEW with formidable sanitizing capabilities. Its antimicrobial effectiveness can be attributed to the synergistic interaction between free available chlorine and the low pH, which disrupts the structural integrity of the cell membranes of pathogens [34]. This mechanism was corroborated in the current investigation, where in AEW (pH 2.5–3) was the only sanitizer among the tested groups, which included chlorine (100 ppm), hydrogen peroxide (1%, 3%), and SAEW (pH 5–6.5) that completely eliminated both *E. coli* and *S. typhimurium* from experimentally contaminated food contact surfaces. Chlorine (100 ppm) achieved merely a 100% reduction of *E. coli*. The enhanced antimicrobial efficacy of AEW, as evidenced herein, underscores its beneficial application in the sanitization of food products.

Recent applications of AEW have encompassed its utilization on meat [35], fish [22] and meat products [33], which is congruent with our prior research endeavors in these areas.

Slightly acidic electrolyzed water (SAEW) is generated through the electrolysis of HCl, whether independently or in conjunction with NaCl, within an electrolytic cell characterized by a pH ranging from 5.0 to 6.5 and an ORP of 800–900 ppm, with its primary active component being HOCl (10–80 ppm), which exhibits a disinfection efficacy 80 times greater than an equivalent concentration of ClO^- under analogous conditions [36]. SAEW is extensively employed in sanitization owing to its high efficacy against a diverse array of pathogens

and its ease of production. Its near-neutral pH renders it a preferred sanitizing agent over AEW due to its diminished corrosive properties. Numerous studies have documented the robust antimicrobial efficacy of SAEW, often employing pure cultures of *E. coli* and *Staph. aureus* to assess its effectiveness in pathogen inactivation [37], and I have personally investigated its application as a sanitizing agent for chilled shrimp [38] and in electrolyzed water-ice (EW-ICE) within the fish industry [22]. In general, sanitizer treatments can reduce bacterial contamination but often fail to achieve complete pathogen elimination. This finding aligns with the results of the current study. Only acidic electrolyzed water (AEW) with a pH of 2–3.5 was able to completely eradicate both *E. coli* and *S. typhimurium*. In this study, sodium hypochlorite (NaClO) at 150 ppm was effective against *E. coli* only. Other treatments, including NaClO at 100 ppm, hydrogen peroxide (H_2O_2) at 1% and 3%, and slightly acidic electrolyzed water (SAEW) with a pH of 5–6.5, were unable to fully eliminate either *E. coli* or *S. typhimurium*. The initial microbial load, treatment conditions, surface adhesion, encapsulation, aggregation, and low-nutrient growth can all have an impact on microbial responses to disinfection. Microorganisms' adhesion or interaction with diverse particulate surfaces might lead to increased disinfection resistance. Thus, prior to selecting and applying a food decontamination method, it is essential to thoroughly understand the target organism and its level of resistance to the chosen technique [39].

Risk assessment, relative log reduction or ratio of effectiveness and Food Safety

Enhancing food safety helps mitigate the risk of foodborne illnesses, leading to more secure and reliable food supplies, which in turn contributes to reducing hunger. Furthermore, it supports the goals of good health and well-being, benefiting both environmental and public health. The risk of *Salmonella* contamination can be substantially reduced by employing suitable disinfectants or sanitizers on food-contact surfaces, thereby significantly enhancing public health [38].

Numerous studies have reported the log reduction of *S. typhimurium* when treated with slightly acidic electrolyzed water (SAEW) and accordingly, the risk level decreases, i.e. the higher the log reduction rate, the higher the safety rate and the lower the risk rate. For instance, Mansur et al. [40] observed a 2.99 log reduction, Rahman et al. [41] reported a 2.3 log reduction, and Al-Holy and Barbara [42] documented a 1.6 log reduction. Deza et al. [43] further demonstrated the efficacy of SAEW in eliminating *Salmonella spp.* as a surface disinfectant on various food processing equipment. These findings align with the results of the current study, where acidic electrolyzed water (AEW) with a pH of 2.5–3.0

completely eradicated *S. typhimurium* from experimentally contaminated food-contact surfaces. Additionally, SAEW in this study achieved a 4.53 reduction rate (79.47%) of the organism.

Increasing public health concerns regarding the use of certain chemical sanitizers, including chlorine, H₂O₂ and others, have prompted bans on these products within the food industry in several European countries. These bans are primarily motivated by the formation of potentially harmful byproducts, such as chloroform, haloacetic acids, trihalomethanes, and chloramines, which are known to be toxic, carcinogenic, and mutagenic [44]. The choice of sanitizers in the food industry involves a multifaceted decision-making process, considering factors such as the target microorganisms, antimicrobial effectiveness, contact time, cost, worker safety, chemical properties, environmental impact, effects on the sensory qualities of food, and compatibility with food processing equipment [45]. Consequently, in this study, we investigated the use of acidic electrolyzed water (AEW) as an innovative, environmentally friendly, cost-effective, and rapidly producible alternative to traditional chemical disinfectants. The goal was to reduce the adverse effects of the previously mentioned chemicals on human health and the environment while demonstrating robust sanitizing effectiveness against

foodborne pathogens contaminating food, food-contact surfaces, utensils, and equipment.

Conclusion

Based on the results and discussion presented in this study, it is clear that acidic electrolyzed water (AEW) with a pH of 2.5–3 demonstrated the highest efficacy as a sanitizer against both *E. coli* and *S. typhimurium*, reducing bacterial counts to below (<1 log₁₀ CFU/g) (a 100% reduction). Sodium hypochlorite (NaClO) at 150 ppm also proved highly effective, achieving a 100% reduction of *E. coli*. As a result, the food industry could consider adopting these disinfectants and sanitizers for the decontamination of food-contact surfaces.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

Not applicable

TABLE A. Type and concentration of disinfectants used in the experiment

Disinfectant used	Concentration
Sodium Hypochlorite (NaClO)	100 ppm
	150 ppm
Hydrogen peroxide (H ₂ O ₂)	1%
	3%
Electrolyzed water (EW) as AEW & SAEW	pH (2-3.5)
	pH (5-6.5)

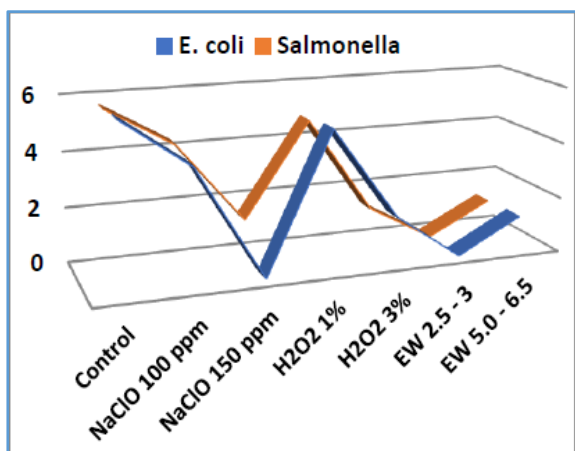
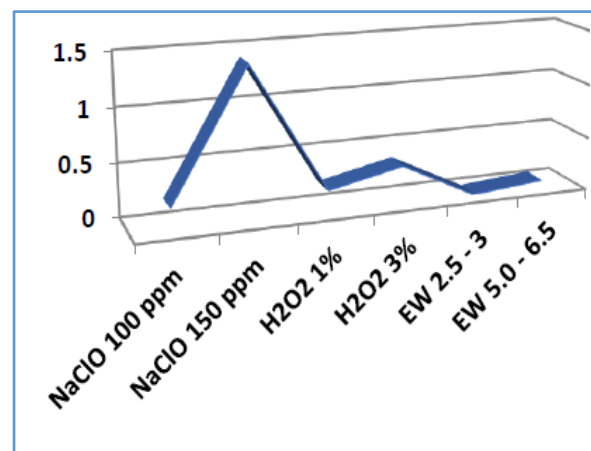
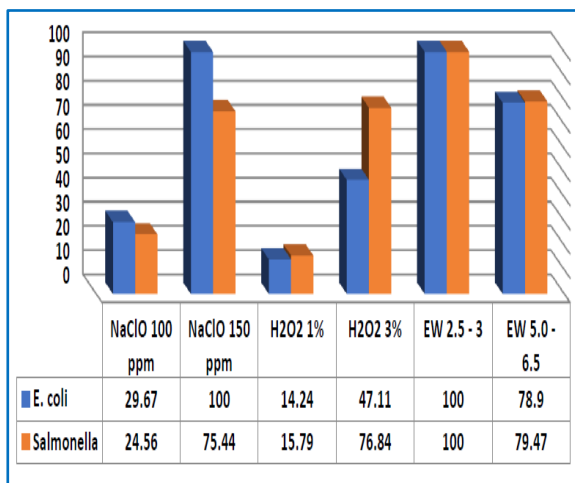
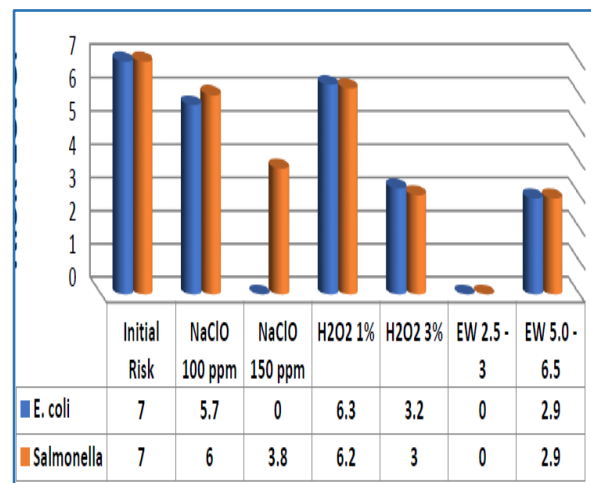
TABLE 1. Statistical analysis of *E. coli* and *Salmonella* (Mean log₁₀cfu) using different sanitizers

Detergent type & concentration		<i>E. coli</i>	<i>S. typhimurium</i>	Log Reduction Scale
		Mean ±SD	Mean ±SD	
Control		5.83 ^a ±0.06	5.70 ^a ±0.10	
Chlorine (Sodium Hypochlorite)	100 ppm	4.1b 0 ^b ±0.10	4.30 ^b ±0.20	0.2
	150 ppm	<1 ^c ±0.00	1.40 ^c ±0.26	1.4
Hydrogen Peroxide (H₂O₂)	1%	5.0 ^d ±0.10	4.80 ^d ±0.10	0.2
	3%	1.67 ^e ±0.15	1.32 ^c ±0.03	0.35
Oxidized Water (AEW)	pH (2.5-3)	<1 ^c ±0.00	<1 ^c ±0.00	0.0
	pH (5.0-6.5)	1.23 ^c ±0.21	1.17 ^c ±0.12	0.06

Detergents (Mean Log₁₀ cfu/cm² ± SD of 3 Trials). Significance differences (P < 0.05) between means having different superscripted small letters within the same column

TABLE 2. The Reduction rate and % (R.R & R%) of Various Disinfectants on *E. coli* and *S. typhimurium*

Detergent type & concentration	Concentration and pH values	<i>E. coli</i>			<i>Salmonella typhimurium</i>		
		Initial <i>E. coli</i>	R.R	R. %	Initial <i>Salmonella</i>	R.R.	R. %
Chlorine (NaClO)	100 ppm	5.83 log ₁₀	1.73	29.67	5.7 log	1.4	24.56
	150 ppm		5.83	100		4.3	75.44
Hydrogen peroxide (H ₂ O ₂)	1%		0.83	14.24		0.9	15.79
	3%		4.16	47.11		4.38	76.84
Oxidized water (AEW)	pH (2.5 – 3)		5.83	100		5.7	100
	pH (5.0 – 6.5)		4.60	78.9		4.53	79.47


Fig. 1. Mean *E. coli* and *S. typhimurium* count

Fig. 2. The Log reduction scale between bacterial mean

Fig. 3. Reduction % *E. coli* and *S. typhimurium*

Fig. 4. Comparative Risk Assessment of *E. coli* and *S. typhimurium* Contamination on Food Contact Surfaces after Disinfection Treatment

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الماء المؤكسد مقابل المطهرات الأخرى: دراسة مقارنة حول التحكم في مخاطر الأسطح الملامسة للطعام الملوثة بالإشريكية القولونية والسالمونيلا

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الملخص

لقد تم إثبات فعالية عدة علاجات في تعقيم الأسطح الفولاذية المقاومة للصدأ الملوثة في المختبر والتي تم تلقيحها بسلالات مرجعية من السالمونيلا والإشريكية القولونية. شملت هذه العلاجات هيبوكلوريت الصوديوم (100 و 150 جزء في المليون)، بيروكسيد الهيدروجين (1% و 3%) والماء المؤين الحمضي (AEW) عند درجة حموضة 2.5-3 والماء المؤين الحمضي (SAEW) عند درجة حموضة 5-6.5. أظهرت أفضل كفاءة من خلال المياه المؤينة الحمضية (AEW) عند درجة حموضة 2.5-3، والتي قضت تمامًا على بكتيريا السالمونيلا والإشريكية القولونية، التي كانت تحمل في البداية أحمالًا بكتيرية تبلغ 5.83 وحدة تشكيل مستعمرة (CFU) والإشريكية القولونية و 5.7 وحدة تشكيل مستعمرة (CFU) لسالمونيلا. بالإضافة إلى ذلك، تم القضاء تمامًا على تلوث الإشريكية القولونية باستخدام هيبوكلوريت الصوديوم بتركيز 150 جزء في المليون. تم تقييم تقليل الحمل البكتيري، وتم حساب مقاييس التقليل جنبًا إلى جنب مع عوامل الخطر (RF) لكل من الإشريكية القولونية والسالمونيلا. كانت مقاييس التخفيف المسجلة: 0.2، 1.4، 0.2، 0.35، 0.0، و 0.06 باستخدام هيبوكلوريت الصوديوم (100 و 150 جزء في المليون)، بيروكسيد الهيدروجين (1% و 3%) والماء الكهربائي الحمضي (AEW) بمدى pH من 2.5-3 و 5-6.5، على التوالي. أدت تقيييمات المخاطر عند 100 جزء في المليون من هيبوكلوريت الصوديوم إلى مستويات عالية من المخاطر (5.7 و 6) لكلا الكائنات. تطبيق 150 جزء في المليون من هيبوكلوريت الصوديوم أدى إلى خطر ضئيل لبكتيريا الإشريكية القولونية (0) ولكنه أدى إلى خطر مرتفع (3.8) لبكتيريا السالمونيلا على العكس، فإن 1% من بيروكسيد الهيدروجين أنتجت مستويات عالية جدًا من المخاطر (6.3 و 6.2) لكلا الكائنات. باستخدام 3% بيروكسيد الهيدروجين كانت مستويات الخطر عالية (3.2) لبكتيريا الإشريكية القولونية ومتوسطة (3) لبكتيريا لسالمونيلا. أظهرت المياه المؤينة الحمضية خطرًا ضئيلًا لكلا الكائنات، بينما أدت المياه المؤينة الحمضية قليلًا إلى مستويات خطر معتدلة (2.9) لكلا الكائنات.

الكلمات الدالة: الماء المُحلل كهربائيًا (AEW و SAEW)، هيبوكلوريت الصوديوم (NaClO)، بيروكسيد الهيدروجين.