Assiut Veterinary Medical Journal Assiut Vet. Med. J. Vol. 71 No. 187 November 2025,

10.21608/avmj.2025.368398.1625 Assiut University website: www.aun.edu.eg

COMPARING THE EFFICIENCY OF ELECTRO ACTIVATED ALKALINE WATER ON FOOD CONTACT SURFACE USING DIFFERENT MINERAL SALTS

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Received: 16 March 2025; **Accepted:** 29 May 2025

ABSTRACT

Surface sanitation is crucial in food industry facilities to prevent the growth and spread of harmful microorganisms that can lead to foodborne illnesses. Alkaline electrolyzed water (ALEW) has emerged as an effective antibacterial agent for surface disinfection, leveraging its high pH and the presence of reactive species like hypochloric acid to eliminate pathogens. Therefore, the current study was planned to evaluate the antibacterial potency of sodium chloride (NaCl), magnesium chloride ($MgCl_2$) and sodium bicarbonate ($NaHCO_3$)-derived ALEW (pH 10±2) as a surface sanitizer against *E. coli* and *S. aureus*. For this goal, stainless steel plates were contaminated with *E. coli* and *S. aureus* strains (nearly counted 4 log CFU/cm^2), followed by application of ALEW for 15 minutes followed by recording the post-treatment counts. Referring to the obtained results, application of ALEW of different salts revealed significant reductions in both of *S. aureus* and *E. coli* mean counts. The obtained results revealed superiority of NaCl-derived EW over $NaHCO_3$ and $MgCl_2$, respectively. Moreover, *S. aureus* was more resistant to the used EW than *E. coli*, where higher reductions were recorded in *E. coli* treated groups. Significant reductions in the bacterial counts were recorded with reductions of 76.1 and 82.2, 65.2 and 71.1, 69.6 and 75.6%; with mean reduction (log CFU/cm^2) of 3.6 and 3.7, 3.0 and 3.2, 3.2 and 3.5 for *S. aureus* and *E. coli* treated groups with NaCl, $MgCl_2$, and $NaHCO_3$-derived ALEW, respectively. Application of ALEW showed potential chance for its usage as potent sanitizer in food processing contact surfaces.

Keywords*:* Electrolyzed water, Food processing, Hygienic quality, Sanitation

INTRODUCTION

In the food industry, maintaining cleanliness and preventing microbial contamination are paramount importance for ensuring food safety and quality (Mainardi and Bidoia, 2024).

Traditional disinfection methods often rely on chemical sanitizers, which can leave residues and pose environmental concerns. So, electrolyzed water (EW) has emerged as a promising alternative, offering a natural, effective, and environmentally friendly solution for surface disinfection (Adal *et al.*, 2024).

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Electrolyzed water is produced by electrolyzing a solution containing dilute salts, such as sodium chloride (NaCl), magnesium chloride ($MgCl_2$), or less commonly, sodium bicarbonate ($NaHCO_3$), in an electrolysis chamber (Yan *et al.*, 2021).

Electrolyzed water has been widely used in the food industry for several years due to its antimicrobial effects against a variety of microorganisms, including bacteria, viruses, spores, and fungi (Mahran and Ibrahim, 2023). It is an effective agent for controlling biofilms and has been shown to be more effective than traditional chlorine solutions in certain applications (AL-Haq *et al.*, 2005). The use of EW offers several advantages, including on-site production, cost-effectiveness, and environmental sustainability. This aligns with the increasing demand for natural sanitizers that minimize chemical residues and environmental impact (Ramchuran *et al.*, 2023).

Alkaline electrolyzed water, typically generated from sodium chloride solutions, is rich in sodium hydroxide (NaOH) and has strong cleaning properties. Although, it is less effective as a disinfectant compared to acidic electrolyzed water, it can still contribute to reducing bacterial loads on surfaces intended for food contact (Tomasello *et al.*, 2021).

Magnesium chloride is another salt that can be used in the production of electrolyzed water. Although less documented than sodium chloride, magnesium chloride could potentially offer similar antimicrobial properties due to the formation of hypochlorous acid during electrolysis. The inclusion of magnesium ions might enhance certain chemical reactions or provide additional benefits, though more research is needed to fully understand its effects in this context (Ampiaw *et al.*, 2021)

Sodium bicarbonate, while not commonly used for electrolyzed water production, might offer unique benefits due to its buffering capacity and potential to enhance certain chemical reactions during electrolysis; however, its application in this area is less well-studied compared to sodium chloride and magnesium chloride (Zang *et al.*, 2019).

As the food industry continues to seek innovative methods for ensuring food safety, the application of electrolyzed water from various salt solutions presents a promising avenue for exploration. Therefore, the current study aimed to evaluate the antibacterial effect of alkaline EW that originated from different salt solutions on some foodborne pathogens as surface decontaminant technique.

MATERIAL AND METHODS

1. Preparation of Alkaline electrolyzed water (ALEW) (Athayde *et al.*, 2018)

Alkaline EW was obtained from Food Hygiene Dept., Animal Health Research Institute. Alkaline EW was prepared in a diaphragm less electrolyzer and has an alkaline pH (10.0±2.0), a relatively low oxidation reduction potential (ORP) (800–900 mV) and a relatively low active chlorine content (ACC) (10–30 mg L−1).

2. Preparation of foodborne pathogens

Field strains of *E. coli* and *S. aureus* were used. The strains were isolated, purified and counted using TBX and Baird Parker agar for *E. coli* and *S. aureus*, respectively. The working count was adjusted at 4 log_{10} CFU/ml.

a. Preparation of stainless-steel surfaces

Ten cm^2 stainless steel plates were sterilized in hot air oven (180°c for 2h), followed by spreading of 4 log CFU/cm *E. coli* and *S. aureus* by means of impregnated cotton swab, separately. Each contaminated plate was left for 10 minutes for dryness before application of EW.

b. Experimental grouping according to Tomasello *et al.* (2021)

Stainless steel plates were grouped as the following:

G1: Control positive for *S. aureus* (~ 4 log CFU/cm^2)

G2: Control positive for *E. coli* (~ 4 log CFU/cm^2)

G3: Contaminated plates with *S. aureus* (~ 4 log CFU/cm^2); which was subgrouped into **G3.1., G3.2., G3.3.** represent treatment with alkaline EW of NaCl, $MgCl_2$, and $NaHCO_3$, respectively.

G4: Contaminated plates with *E. coli* (~ 4 log CFU/cm^2); which was subgrouped into **G4.1., G4.2., G4.3.** represent treatment with alkaline EW of NaCl, $MgCl_2$, and $NaHCO_3$, respectively.

After drying, samples were taken from control groups by sterile cotton swabs for counting the bacterial load. In treated groups, alkaline EW was then applied with a pump, left to dry for about 15 minutes, each plate was sampled by sterile cotton swab, to evaluate the bacterial load after treatment. Swab sampling and preparation were conducted following the instructions of ISO 18593 (2018). 0.1 mL of the sample obtained were plated, incubated and counted according to ISO 16649-2 (2001) and ISO 6888-1 (2021) for *E. coli* and *S. aureus* counting, respectively. The trial was repeated in triplicates.

c. Statistical analyses were performed by application of paired T-test and one-way ANOVA test on SPSS software v.20. Reduction (%) = $\left(\frac{(R1-R2)}{R1}\right) x\ 100$, where R1 and R2 indicate microbial count of control and treated samples, respectively. Results were expressed as log CFU/cm^2, and Duncan post-hoc test ($p \leq 0.05$).

RESULTS

Referring to the obtained results, application of alkaline EW of different salts revealed significant reductions for both of *S. aureus* and *E. coli* man counts. The obtained results revealed superiority of NaCl-derived EW over $NaHCO_3$ and $MgCl_2$, respectively as it gave higher antibacterial effect. Moreover, *S. aureus* was more resistant to the used EW than *E. coli*, where higher reductions were recorded in *E. coli* treated groups.

Table (1) showed significant reductions in *S. aureus* counts with reduction (%) of 76.1, 65.2 and 69.6 with mean reduction (log CFU/cm^2) of 3.6, 3.0 and 3.2 in G3.1, G3.2 and G3.3, respectively.

Table 1: *Staphylococcus aureus* mean counts (log CFU/cm^2) before and after treatment with alkaline EW, microbial reduction (mean ±SD), and reduction %

	control group	**Post-treatment**	**Mean reduction (log ± SD)**	**Reduction %**	***P* value (independent-samples T test)**
NaCl alkaline EW (G3.1.)	4.6 ± 0.1^a	$1.1\pm0.0.05^c$	3.6±0.1	76.1	< 0.001
$MgCl_2$ alkaline EW (G3.2.)	4.6 ± 0.1^a	1.6 ± 0.1^a	3.0±0.1	65.2	< 0.001
$NaHCO_3$ alkaline EW (G3.3.)	4.6 ± 0.1^a	1.4 ± 0.1^b	3.2±0.1	69.6	< 0.001
***P* value (ANOVA)**	0.872	0.003			

abc: different superscript letters in the same column means significant difference ($P \leq 0.05$) using ANOVA statistical test

*-. Superscript star between pre- and post-treatment within the same group means significant difference ($P \leq 0.05$) using independent samples test.

On the other hand, Table (2) showed significant reductions in *E. coli* counts with reduction (%) of 82.2, 71.1 and 75.6 with mean reduction (log CFU/cm^2) of 3.7, 3.2 and 3.5 in G4.1, G4.2 and G4.3, respectively.

Table 2: *Escherichia coli* mean counts (log CFU/cm^2) before and after treatment with alkaline EW, microbial reduction (mean ±SD), and reduction %.

	Pre-treatment (control group)	Post-treatment	Mean reduction (log ± SD)	Reduction %	*P* value (independent-samples T test)
NaCl alkaline EW (G4.1.)	4.5±0.3^a	0.8±0.1^c	3.7±0.2	82.2	< 0.001
$MgCl_2$ alkaline EW (G4.2.)	4.5±0.3^a	1.3±0.1^a	3.2±0.2	71.1	< 0.001
$NaHCO_3$ alkaline EW (G4.3.)	4.5±0.3^a	1.1±0.1^b	3.5±0.3	75.6	< 0.001
***P* value (ANOVA)**	1.000	< 0.001			

abc: different superscript letters in the same column means significant difference ($P \leq 0.05$) using ANOVA statistical test

*-. Superscript star between pre- and post-treatment within the same group means significant difference ($P \leq 0.05$) using independent samples T test.

DISCUSSION

Surface sanitation, food hygiene, and the use of environmentally safe disinfectants are critical components in the food industry, ensuring the safety and quality of food products (FAO-WHO, 2011). Effective surface sanitation helps prevent microbial contamination, which can lead to foodborne illnesses and compromise product integrity (Tropea, 2022). Regular cleaning and sanitizing of food-contact surfaces, such as cutting boards, countertops, and processing equipment, are essential practices that reduce the presence of harmful pathogens like Salmonella and *E. coli*. Stringent sanitation protocols may protect consumers from potential health risks while maintaining their reputation for quality and safety (Lai *et al.*, 2023).

During the production of alkaline electrolyzed water, electrolysis involves splitting water into hydrogen and oxygen using an alkaline electrolyte solution, typically potassium hydroxide (KOH) or sodium hydroxide (NaOH) (Chatenet *et al.*, 2022). This process occurs in an electrochemical cell with two electrodes: an anode where oxygen is released and a cathode where hydrogen is produced. Although the primary purpose of this process is not bactericidal, the resulting alkaline stream can have some indirect effects on microbial environments (Carmo *et al.*, 2013). However, its bactericidal effect is generally less pronounced compared to acidic electrolyzed water, which contains hypochlorous acid—a potent antimicrobial agent. The cleaning effect of alkaline electrolyzed water might help reduce microbial loads by physically disrupting biofilms rather than direct chemical action against bacteria (Schalenbach *et al.*, 2016).

Alkaline electrolyzed water (ALEW) has gained significant attention in the food industry as an effective and environmentally friendly surface disinfectant as well as its ease for on-site production and potent antimicrobial activity (Tomasello *et al.*, 2021). Its application is primarily focused on cleaning food-contact surfaces, such as stainless steel and glass, which are commonly used in food processing facilities (Khalid *et al.*, 2021).

Referring to the obtained results, application of ALEW of different salts revealed significant reductions in both *S. aureus* and *E. coli* mean counts; which came in agree with the recorded results of Yan *et al.* (2021), Tomasello *et al.* (2021), Rebezov *et al.* (2022) and Guo *et al.* (2024) who emphasized that ALEW is effective against a range of foodborne pathogens, including *L. monocytogenes, Salmonella, E. coli* and *S. aureus*. The studies noted that ALEW can significantly reduce microbial loads on food contact surfaces, such as stainless steel and plastic, thereby enhancing food safety.

Reductions in the bacterial counts is primarily attributed to the alkaline pH of the applied ALEW; as well as, the oxidative properties of hypochlorous acid (HOCl) and other active chlorine generated during electrolysis, which disrupt bacterial cell membranes and metabolic functions (Chen and Wang, 2022).

While alkaline electrolyzed water does not possess the same level of direct antibacterial potency as acidic forms due to differences in pH-dependent active compounds, it offers benefits through a washing effect rather than direct bactericidal action. It can disrupt biofilm structures by removing extracellular polymers, which helps in reducing microbial loads on surfaces, besides that, its effectiveness may stem from physical removal or disruption rather than chemical destruction (Liu *et al.*, 2023).

ALEW is a well-known detergent that dissolves proteins and lipids. The cleaning action of ALEW can increase the disinfectant's efficacy by eliminating organic debris that could harbor germs, as it has been proposed that employing ALEW as a pre-treatment before applying disinfectants might boost their action (Jiménez-Pichardo *et al.,* 2016).

In particular, ALEW demonstrated considerable ($P<0.05$) efficacy against *S. aureus* and *E. coli*. The average microbial reduction were of 3.7, 3.2, and 3.5 log CFU/ cm^2 for NaCl, $MgCl_2$, and $NaHCO_3$ derived EW, respectively, it seemed to be significantly ($P \leq 0.05$) more effective against Gram negative bacteria (*E. coli*) than Gram positive bacteria (*S. aureus*), with average bacterial reductions of 3.6, 3.0, and 3.2 log CFU/cm^2 for NaCl, $MgCl_2$, and $NaHCO_3$ derived EW, respectively.

In addition, the thickness of the cell wall, which maintains the integrity of the cell, is the reason why Gram positive bacteria are more resistant to sanitizing treatments than Gram negative bacteria, according to a wealth of evidence found in various records (Mai-Prochnow *et al.*, 2016 and Tomasello *et al.*, 2021).

Gram positive bacteria exhibited comparatively greater resilience to the treatment than Gram negative bacteria, according to comparable findings by other authors studying slightly acidic electrolyzed water (SAEW) (Tango *et al.*, 2015 and Hamed *et al.*, 2024).

Moreover, in the current study, ALEW derived from NaCl electrolysis showed higher antibacterial effect against *S. aureus* and *E. coli* than those of $MgCl_2$ and $NaHCO_3$ which may be attributed to the presence of hypochloric acid (HOCl) and hypochlorite ions, which can penetrate bacterial membranes and disrupt cellular functions (Vahabi *et al.*, 2020). However, the antibacterial properties of $MgCl_2$ alone are not as well-documented, but some studies suggest that magnesium ions could improve the overall antimicrobial action when used in combination with other electrolytes (Demishtein *et al.*, 2019).

Previous studies indicated that $MgCl_2$ electrolyzed water exhibits significant antimicrobial properties against various foodborne pathogens. For instance, Yan *et al.* (2021) demonstrated that electrolyzed water produced from magnesium chloride effectively reduced the microbial load of *E. coli* and Salmonella on food contact surfaces. The study highlighted that the mechanism of action involves the disruption of bacterial cell membranes, leading to cell lysis and death. This was attributed to the ionic strength and specific interactions of magnesium ions with bacterial structures, which enhance the permeability of cell membranes and promote leakage of intracellular components, ultimately resulting in bacterial inactivation.

However, magnesium chloride was revealed to have a lower inhibitory effect than NaCl-derived EW on the examined bacteria, sodium bicarbonate derived EW revealed significant (P≤0.05) reduction in the bacterial counts, which may be attributed primarily to altering the pH environment, which can inhibit bacterial growth (Vahabi *et al.*, 2020). While effective against certain pathogens, their standalone efficacy is generally lower compared to NaCl-based solutions (Santoyo *et al.*, 2024).

It is worth noting that the major mechanism of action of alkaline electrolyzed water (ALEW) primarily involves the generation of reactive species, particularly hypochlorous acid (HOCl) and hydroxyl radicals (OH·), during the electrolysis of a saline solution. In this process, hydroxide ions (OH^-) are produced at the cathode (Chen and Wang, 2022). The hypochloric acid is a potent oxidizing agent that can penetrate bacterial cell membranes due to its small size and neutral charge. Once inside the cell, HOCl disrupts essential cellular processes by denaturing proteins, damaging nucleic acids, and altering membrane permeability, ultimately leading to microbial inactivation. The high pH of ALEW enhances its cleaning properties while also promoting the formation of active species that disrupt bacterial metabolism and cell integrity (Liu *et al.*, 2023). Factors such as pH, oxidation-reduction potential (ORP), and available chlorine concentration significantly influence the effectiveness of ALEW in various disinfection applications within the food industry (Adal *et al.*, 2024).

CONCLUSION

Referring to the obtained results, application of alkaline EW of different salts revealed significant reductions in both of *S. aureus* and *E. coli* man counts. However, the obtained results revealed superiority of NaCl-derived EW over $NaHCO_3$ and $MgCl_2$, they revealed potent antibacterial effects as well. Moreover, *S. aureus* was more resistant to the used EW than *E. coli*, where higher reductions were recorded in *E. coli* treated groups. So, it can be recommended to apply ALEW on large scale as food-contact-surface sanitizer.

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مقارنة كفاءة الماء القلوى النشط كهربائياً على الأسطح الملامسة للغذاء باستخدام أملاح عضوية مختلفة

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يُعد تطهير الأسطح أمرًا بالغ الأهمية في مرافق صناعة الأغذية لمنع نمو وانتشار الكائنات الحية الدقيقة الضارة التي يمكن أن تؤدي إلى أمراض التسمم الغذائي. وقد أظهرت المياه القلوية المحللة كهربائيًا تأثيراً فعالاً مضاد للبكتريا والتي يمكن تطبيقها لتطهير الأسطح، مستفيدة من درجة الحموضة العالية ووجود بعض المركبات مثل حمض الهيبوكلوروس للقضاء على الميكروبات المسببة للأمراض. لذا، أجريت الدراسة الحالية لتقييم الفعالية المضادة للبكتريا للمحلول القلوي للماء المحللة كهربياً المشتقة من تأين أملاح كلوريد الصوديوم وكلوريد المغنيسيوم وبيكربونات الصوديوم (درجة الحموضة ١٠ ± ٢) كمطهر سطحي ضد بكتريا الايكولاي والاستافيلوكوكس اوريس. ولتحقيق ذلك، تم تلويث ألواح الاستانلس ستيل ببكتريا الايكولاي والاستافيلوكوكس اوريس بمتوسط عدد ٤ لو.١٠ خلية/سم٢ تقريباً، ثم تم تطبيق المياه القلوية المحللة كهربائيًا لمدة ١٥ دقيقة وتسجيل أعداد البكتريا بعد المعالجة. بالإشارة إلى النتائج التي تم الحصول عليها، أظهر تطبيق المياه القلوية المحللة كهربائيًا والمشتقة من مختلف الأملاح محل الدراسة انخفاضاً معنوياً في العد البكتيري للبكتريا محل الدراسة. كشفت النتائج التي تم الحصول عليها عن تفوق المياه القلوية المحللة كهربائيًا المشتقة من كلوريد الصوديوم على تلك المشتق من بيكربونات الصوديوم وكلوريد المغنسيوم، على التوالي. علاوة على ذلك، أظهرت بكتريا الاستافيلوكوكس اوريس مقاومة أكبر من الايكولاي تجاه المياه القلوية المحللة كهربائيًا اثناء الدراسة، حيث تم تسجيل انخفاضاً اكبر في اعداد الايكولاي بنسبة انخفاض (%) بلغت ٧٦,١ و٨٢,٢ و٦٥,٢ و٧١,١ و٦٩,٦ و٧٥,٦٪؛ مع متوسط انخفاض (لو.١٠ خلية/سم٢) بلغ ٣,٦ و٣,٧ و٣,٠ و٣,٢ و٣,٢ و٣,٥ في مجموعات الاستافيلوكوكس اوريس والايكولاي المعالجة بالمياه القلوية المحللة كهربائيًا المشتقة من أملاح كلوريد الصوديوم وكلوريد المغنسيوم وبيكربونات الصوديوم، على التوالي. مما سبق يمكن التوصية باستخدام المياه القلوية المحللة كهربائيًا لتطهير الاسطح المتصلة بالغذاء واستخدامها كمطهر قوي آمن علي البيئة وصحة المستهلكين.

الكلمات الدالة: الماء المحلل كهربائياً، سلامة الأغذية، التطهير