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Assessment of the Efficiency of Electrolyzed Water in Controlling The contamination of Fish Fillets With *V. parahaemolyticus*

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Abstract

NOWADAYS, electrolyzed water (EW) is widely identified as a substitute for chemical antimicrobials to decrease microbial contaminations and extend the food shelf life. In this work, tilapia fish fillets that have been intentionally contaminated with *Vibrio parahaemolyticus* are used to examine the antibacterial effects of slightly acidic (SAEW) and neutral electrolyzed water (NEW) with two amounts of NaCl (0.2% and 0.5%) individually. The study also examines their influence on the quality and sensory properties of tilapia fish and the expression of virulence genes tdh, trh, and toxR using qRT-PCR. Samples of tilapia fish fillets were artificially infected with *V. parahaemolyticus*, then followed by immersion separately in SAEW and NEW (0.2 % and 0.5 % NaCl) for 2, 5 and 10 minutes at ambient temperature, afterward the samples were retained in a refrigerator at 4±1°C. Results showed that *V. parahaemolyticus* counts on the 3rd day of storage were reduced with NEW and completely inhibited with SAEW. Additionally, the shelf life of all treated fillet specimens was prolonged to the 7th and 9th day by slowing the deterioration of odour and colour compared to the untreated samples, which became unfit for consumption. EW specifically, SAEW containing 0.5% NaCl showed better physicochemical characteristics. There was also a significant decrease in the virulence gene expression between the control untreated and the other treated samples. In conclusion, electrolyzed water can be applied as a sterilizer to improve microbic attributes and increase the shelf life of fish fillets.

Keywords: *V. parahaemolyticus*, Tilapia fish fillet, Electrolyzed water, qRT-PCR, Shelf life**.**

Introduction

Seafood contains necessary nutrients such as high-quality amino acids, omega-3 fatty acids, minerals like phosphates and calcium, and various vitamins [1]. However, seafood can be vulnerable to bacterial diseases, impacting its quality and safety, and leading to foodborne illnesses. *Vibrios* are among the most prevalent foodborne bacteria on water surfaces and are associated with cases of food poisoning [2].

The most common pathogenic *Vibrio* species associated with human utilization of undercooked or raw fish are *Vibrio vulnificus, Vibrio parahaemolyticus*, and *Vibrio alginolyticus* [3]. *Vibrio parahaemolyticus* infection usually occurs when consuming raw or undercooked seafood contaminated with this bacterium. This can lead to severe gastroenteritis, characterized by watery stools, fever, chills, diarrhea, nausea, vomiting, and stomach pain [4].

The toxR gene, which plays a role in the cytotoxicity and hemolytic behaviour of *V. parahaemolyticus* in host cells, reinforces the understanding of the pathogenicity of this bacterium in the context of seafood [5]. Thermostable direct haemolysin (TDH) and TDH-related haemolysin

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(TRH) represent two principal virulence factors of *V. parahaemolyticus*, which are intricately linked to its pathogenic potential. Both factors exhibit comparable haemolytic activity in vitro, leading to the lysis of human erythrocytes in a highly saline environment [6]. TDH binds to the membranes of host cells or erythrocytes, escorting to the formation of a hole on the membrane's surface that facilitates the passage of red blood cell colloids. Additionally, TDH exhibits cytotoxic properties; it inflicts damage on cells and establishes a channel within the cell membrane, resulting in elevated levels of extracellular Ca2+ and enhanced secretion of Cl- [7].

A range of sanitization techniques has been implemented to enhance the quality and safety of fresh meat, fish, poultry, and meat products [8]. Researchers are investigating alternative approaches to traditional chemical sanitizers, including chlorine, dihydrogen dioxide, and peracetic acid, for the sanitization of fish, poultry, fresh meat, and vegetables [9].

Electrolyzed Water (EW) is an eco-friendly popular disinfectant, sanitizing agent, and antimicrobial agent in the food industry [10]. It is generated from distilled water and NaCl. It has many advantages as it is safe for the environment, avoids the problems of chlorination during transport, storage, and handling, and has no harmful effects on human health [11]. It effectively decontaminates and preserves food by fighting off various food borne pathogenic bacteria like *E. coli O157:H7, L. monocytogenes, S. typhimurium, S. aureus,* and *V. parahaemolyticus* [12]. EW comes in three types: Acidic, Neutral, and Alkaline, each with antimicrobial characteristics and the capability to eliminate a range of pathogenic organisms [13].

SAEW, characterized as a mildly acidic electrolyzed water, possesses a significant amount of hydroxidochlorine, exhibiting a pH ranging from 5.0 to 6.5, which helps minimize surface corrosion of fresh products and reduces potential environmental and human health damage. Research shows that electrolyzed water combined with mild heating is more effective in reducing harmful organisms in food and extending the freshness of aquatic products and vegetables [24].

NEW exhibits an approximately neutral pH value, ranging from 7 to 8. This results in a similar antimicrobial effect and less surface corrosion and skin irritation compared to Acidic Electrolyzed Water (AEW). NEW is more stable during storage and has been widely used for sterilization and inactivating food-borne bacteria [10].

Shelf life is defined as the interval in which a food item or product can be safely consumed while preserving its appropriate microbiological, physicochemical, and sensory characteristics [15]. This research aimed to estimate the antimicrobial effects of slightly acidic and neutral electrolyzed water on tilapia fish fillets contaminated with *V. parahaemolyticus*. Additionally, the study examined these treatments' impact on the fish fillets' shelf life and their effect on the virulence gene expression, as measured by qRT-PCR.

Material and Methods

Preparation of V. parahaemolyticus bacterial strains:

The *Vibrio parahaemolyticus* strain (NCTC 10885) was obtained from the Reference Laboratory for Food Safety at the Animal Health Research Institute in Dokki, Egypt. The strain was stored on tryptic soy agar slants containing 3% NaCl at 4°C. Before the experiment, fresh microbial cultures were adjusted to 0.5 McFarland, which is approximately equal to 8 log10 CFU/ml [16].

Electrolyzed Water Preparation

According to Athayde et al. [17], the preparation of slightly acidic and neutral electrolyzed water (SAEW and NEW) begins with the use of potable drinking water, to which sodium chloride (NaCl) is added at levels of 0.2% and 0.5%, respectively. An electrolysis cell is employed, through which a current of 9-10 volts is passed, utilizing anodes (+) and cathodes (-). Through the electrolysis process, NaCl dissociates into sodium (Na^+) and chloride (Cl^-) ions, while water undergoes reduction at the cathode, resulting in the formation of hydroxide (OH^-) and hydrogen (H^+) ions. The negatively charged ions (OH^- and Cl^-) migrate towards the anode, leading to the production of hypochlorous acid (HOCl), hypochlorite ions (OCl^-), oxygen gas (O_2), and chlorine gas (Cl_2). Conversely, the positively charged ions (Na^+ and H^+) move towards the cathode, yielding sodium hydroxide (NaOH) and hydrogen gas (H_2). To achieve a pH of 5.5 for SAEW, a few drops of 5% vinegar are added, while NEW is maintained at a pH level near 7. Finally, both SAEW and NEW should be labeled and stored in sealed glass containers at a refrigeration temperature of 4°C.

Samples

A total of 1500 grams of tilapia fillets were procured from retail outlets in Tanta, located in the Gharbia Governorate of Egypt. The fillets were subsequently transported to the laboratory in a protected ice box, ensuring sterile conditions were maintained throughout the process. Upon arrival, the fillets were partitioned into six groups, each weighing 250 grams, and were placed in disposable food packaging trays constructed from polypropylene.

Experimental procedure

All groups except the control -ve group (G0) were dipped in a *V. parahaemolyticus* suspension with a level of 10^8 CFU/mL and kept in the refrigerator for 30 minutes to allow attachment. The

initial *V. parahaemolyticus* load was enumerated before treatment [18]. Subsequently, all samples were removed and dried. The control –ve group (G0) and the control + ve group (G1) were submerged in distilled water (DW), while the second and third groups were submerged in SAEW with 0.2% NaCl and pH 5.8, and SAEW with 0.5% NaCl and pH 5.6, respectively. The fourth and fifth groups were immersed in neutral electrolyzed water (NEW) with 0.2% NaCl and pH 7.4, and NEW with 0.5% NaCl and pH 7, respectively as demonstrated in Table (1).

Microbiological examination

The *V. parahaemolyticus* count of treated and untreated fish fillet samples was determined immediately after dipping them in a *V. parahaemolyticus* suspension to establish the initial load before treatment. The count was taken after 2, 5, and 10 minutes of immersion in the treatment solution, and subsequently, after the 1st, 3rd, 5th, 7th, and 9th day intervals until spoilage occurred. A total of ten grams from each sample were accurately weighted underneath sterile provisions and subsequently placed into sterilized "Stomacher" bags for bacteriological inspection. Following this, 90 mL of sanitary physiological saline containing 3% NaCl was introduced, and then two minutes of homogenization. A ten-fold serial dilution was prepared, and 0.1 ml from each dilution was spread onto the selective medium Thiosulfate citrate bile salt sucrose agar (TCBS). These plates were then incubated for 24 hours at 37°C. The colonies of *V. parahaemolyticus* appear round, (measuring 2-3 mm in diameter, and displayed a bluish-green on the selective medium. This entire experiment was performed in triplicate [19].

Sensory assessment

The evaluation was conducted by a panel of ten seasoned experts from the Food Hygiene Department at the Animal Health Research Institute, Tanta Branch. Each group was assessed according to criteria such as color, texture, odor, and overall acceptability, adhering to the modified guidelines established by Bai et al. [20]. A higher score reflected superior quality, whereas a lower score denoted inferior quality. The sensory assessments utilized a 5-point hedonic scale, with ratings ranging from 1 (poor) to 5 (excellent).

Physicochemical evaluation

Determination of pH: it was assessed using a pH meter (Testo AG205, Germany) by placing an electrode into a fish fillet sample (taking the mean of six readings) [21].

Determination of Thiobarbituric acid value (TBA): it was established using the technique described by Buege and Aust [22]. The samples were homogenized with a TBA reagent composed of 250 mM/L HCl, 15% w/v TCA, and 0.375% w/v TBA in a ratio of five volumes to one. The resulting mix was heated for 10 minutes, afterward centrifugated at 4°C for 25 minutes at 4500 rpm, after which the absorbance was recorded at a wavelength of 532 nm.

Determination of total volatile basic nitrogen (TVBN): It was determined by the assay of Huang et al. [21]. The minced sample, weighing 3 g, was mixed with 100 mL of ultrapure water and allowed to blend for 30 minutes. After this initial mixing period, 1 g of magnesium monoxide was added to the solution. The measurement of TVBN was conducted using a Kjeltec™ 9 Distillator (FOSS, Denmark), with results expressed as mg TVBN per 100 g of fish meat.

The TVBN concentration was calculated with the following equation:

$$\text{TVBN (mg/100 g)} = \frac{(V1 - V2) \times C \times 14}{m\frac{3}{100}} \times 100$$

V1 (mL) is the volume of hydrochloric acid administered to the sample groups, and V2 (mL) is the volume used for the blank group. The variable C stands for the concentration of hydrochloric acid in mol/L, and m represents the weight of the samples in grams.

Revelation of virulence genes

a- Revelation of trh, tdh, toxR virulence genes before treatment

DNA extraction. Extraction of DNA was done by using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications. Oligonucleotide Primers used were from Metabion (Germany) and are listed in Table (2). For PCR amplification, we utilized the Primers in a 25- µl reaction containing 12.5 µl of EmeraldAmp Max PCR Master Mix (Takara, Japan), The reaction was done in a 2720 thermal cycler.

For PCR analysis, the PCR products were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer, 100 bp DNA Ladder (Fermentas, Thermo Scientific, Germany) was used for detection of the fragment sizes.

b- Revelation of gene expression after treatment

RNA extraction from samples was done using QIAamp RNeasy Mini kit (Qiagen, Germany, GmbH). The Oligonucleotide Primers from Metabion (Germany) which are listed in Table (3). We utilized Primers in a 20- µl reaction containing 10 µl of the 2x *HERA* SYBR® Green RT-qPCR Master Mix (Willowfort, UK), The reaction was done by using a step one real-time PCR machine in the Biotechnology Unit, Animal Health Research Institute, Zagazig Branch, Egypt.

Analysis of the results of SYBR green rt-PCR by determination of amplification curves and Ct values

was done by the Step One software. To estimate the gene expression variation among the RNA samples, the Ct of each sample was compared with that of the positive control group using the "ΔΔCt" method [25]. Then calculate by using the following ratio: 2^(-ΔΔCt).

Statistical analysis

The records underwent analysis through one-way ANOVA in SPSS (Version 20). RT-PCR data was processed using Microsoft Excel. Duncan's multiple-range test was employed to conduct multiple mean comparisons [28]. a *P-value* of less than 0.05 indicated significance.

Results

V. parahaemolyticus count

The mean count of *V. parahaemolyticus* after dipping tilapia fish fillet samples in a solution containing *V. parahaemolyticus* at 8.35 log10 CFU/mL for 30 minutes was about 6.53 log10 CFU/gram. The results in Table (4) showed the mean *V. parahaemolyticus* count (log10 CFU/g) of the control group (G1) and treated tilapia fish fillet samples with SAEW and NEW with 0.2% and 0.5% NaCl. Increasing immersion time resulted in more reduction in *V. parahaemolyticus* count. Moreover, SAEW has better count reduction.

Sensory evaluation

The sensory scores for both the control and immersed samples in SAEW and NEA (0.2% and 0.5% NaCl) for about 10 minutes declined significantly with an increasing storage period. The result shown in Figure (1) indicated that all treated groups maintained better sensory quality than the untreated groups.

Physicochemical characteristics

pH values

The pH levels during the storage period for the six experimental groups are detailed in Table 5, showing a considerable enhancement observed with an extended storage period. On the third day, the control positive group (G1) demonstrated a significant rise in pH in comparison to the negative control group (G0) and the groups subjected to EW treatment.

Thio barbituric acid value (TBA)

The TBA levels of the six groups kept at 4°C are shown in Table 6, indicating a consistent increase over time. SAEW with 0.5% NaCl leads to the lowest TBA concentration.

Total volatile basic nitrogen (TVBN)

The concentrations of TVBN in the fish fillet samples from six groups were measured while they were stored at 4°C (Table 7). The TVBN amounts increased gradually in all groups during the storage period. It is worth noting that the group treated with 0.5% NaCl in SAEW exhibited the lowest TVBN value.

The expression of virulence genes (tdh, trh, and toxR) using qRT-PCR

The study found that the intervention had a significant effect on the gene expression of the under investigation. There were no significant changes in the tdh and toxR genes between the control group and G2 ($p > 0.05$). However, there were noticeable differences between G1 and the other treatment groups ($p < 0.05$), with G5 showing the lowest level of expression. The transcription of the trh gene was substantially diminished in all treated samples compared to the G1 ($p < 0.05$), with G3 showing the lowest expression. As seen in Table 8 and Figure 2, no significant variations were found between G4 and G5 ($p > 0.05$).

Discussion

Vibrio parahaemolyticus is a prevalent foodborne pathogenic bacterium that is commonly related to a range of seafood and aquatic environments, representing a significant threat to public health [29]. Electrolyzed water (EW) is widely used in the food industry as an antimicrobic agent against pathogens in foods like chicken, shrimp, meat, fish, and eggs [30]. EW effectively disinfects food and reduces the count of pathogens such as *E. coli*, *S. typhimurium*, *L. monocytogenes*, *S. aureus*, and *V. parahaemolyticus* [12]. Our study showed that immersion of the samples for about 2 minutes resulted in reducing the count of *V. parahaemolyticus* from 6.53 log10 CFU/g of the control to 4.67±0.1 for G3 followed by 4.67±0.1 log10 CFU/g for G2, then 4.87±0.3 and 5.01±0.2 log10 CFU/g for G5 and G4 respectively. There were no alterations ($P>0.05$) between G1 and G4. Meanwhile, after immersion for 5 minutes, significant variances existed ($P<0.05$) between the control positive group G1 (4.91 ± 0.07 log10 cfu/g) and only the samples treated with SAEW 0.2% and 0.5% NaCl (G2 and G3) 3.99 ± 0.08 and 3.87 ± 0.09 log10 CFU/g, respectively. With further increased immersion for 10 minutes, there were clear variances ($P<0.05$) between G1 (4.82 ± 0.3 log10 CFU/g) and all treated groups G2, G3, G4, and G5 (2.06 ± 0.05, 1.76 ± 0.2, 2.94 ± 0.05, and 2.67 ± 0.3 log10 CFU/g), respectively. After 24 hours, complete inhibition was observed in G3, while significant reductions were seen in G2, G4, and G5. After that, the *V. parahaemolyticus* count slightly increased, but the lowest count was recorded in G3 and G2 (4.28 ± 0.5 and 4.58 ± 0.3 log10 CFU/g), respectively, on the 9th day of storing. These findings are consistent with preceding studies that showed the effectiveness of EW in inhibiting *V. parahaemolyticus* growth in seafood [25,31,32]. Yuan et al. [33] stated that the

NEW effect increases with more time and available chlorine concentration (ACC).

The reduction in *V. parahaemolyticus* counts was more pronounced with SAEW compared to NEW. This discrepancy can be attributed to the unique characteristics of the electrolyzed water types, as well as variations in pH, ACC, and ORP levels. These parameters elucidate the bactericidal properties of SAEW, which arise from the synergistic effects of ACC, ORP, and pH [34]. The generation of Hydrogen hypochlorite (HOCl) and hypochlorite ion (OCl-) is influenced by the solution's pH. Specifically, elevated pH levels favor the production of OCl-, while lower pH levels yield a combination of chlorine (Cl2) and HOCl. At a neutral pH range of 7 to 8, HOCl predominates, achieving optimal concentration with minimal dissociation [35]. HOCl is capable of penetrating cell membranes and generating hydroxyl radicals that target microbial cells. These radicals exert antimicrobial effects by oxidizing essential metabolic pathways. The ratios of chlorine species (HOCl, Cl_2, and OCl-) are contingent upon pH levels, which in turn influence the bactericidal efficacy of AEW [36]. The peak concentration of HOCl correlates with the highest effectiveness of AEW in bacterial inactivation, particularly at pH levels between 4.0 and 5.0. Furthermore, the characteristics of electrolyzed water are influenced by various factors, including electrode materials, salt concentration, storage conditions, and water temperature [37]. Huang et al. [38] demonstrated that SAEW exhibits significant bactericidal activity, effectively inhibiting the growth of food spoilage bacteria on food surfaces, as shrimp. Wang et al. [39] noted that SAEW, characterized by zero salinity and a pH close to 6, creates an inhospitable environment for *V. parahaemolyticus* proliferation. Additionally, both SAEW and NEW have been shown to possess robust bactericidal properties against a range of foodborne pathogens and spoilage microbes on various food products and equipment surfaces [40].

The count of *V. parahaemolyticus* tends to rise over extended storage durations, which can be attributed to a reduction in EW activity. The efficacy of EW diminishes when exposed to organic substances, including amino acids and proteins [41]. In electrolyzed water, free chlorine interacts with these organic compounds, resulting in the formation of organo-chloramines. It is noteworthy that the bactericidal effectiveness of combined available chlorine is inferior to that of its free counterpart [42].

Lipid peroxidation is a consequence of the oxidative degradation of polyunsaturated fatty acids present in muscle tissue, leading to the formation of undesirable odors and flavors. This phenomenon negatively impacts the shelf-life of seafood [43]. The sensory evaluations for all treated groups, encompassing aspects such as color, odor, texture, and overall acceptability, were significantly superior to those of the control group ($p < 0.05$). The control positive group (G1) displayed indications of spoilage by the third day, whereas the control negative group (G0) remained sound until the fifth day of storage. Notably, on the seventh day, group G3 achieved a higher sensory score compared to the other groups ($p < 0.05$). Furthermore, G2, G4, and G5 maintained their natural properties and remained fit for human consumption during the storage period. The samples were considered unacceptable for consumers upon spoilage throughout the storage period, even though the microbial load did not exceed the permissible limit [44].

The application of electrolyzed water (EW) substantially prolonged the shelf life of fish fillets by effectively delaying quality degradation, which resulted in improved sensory evaluations. Zang et al. [45] indicated that strong acid-electrolyzed water serves as a sanitizer that diminishes microbial contamination, thereby enhancing the shelf life of aquatic products. This finding is consistent with the research conducted by Iram et al. [10], who demonstrated that SAEW could significantly prolong the shelf life of beef and chicken when compared to alternative preservation methods. Furthermore, Cen et al. [46] noted that tilapia fillets treated with SAEW experienced an extension of shelf life by 3 to 4 days, and also maintained superior quality. Additionally, the use of neutral electrolyzed water has the potential to promote consumer health by lowering bacterial counts on fish samples and extending their shelf life [47].

In our study, we noted a marked elevation in pH on the third day within the G1 samples when compared to the treated groups. This elevation can be attributed to the exhaustion of energy reserves alongside the lactic acid accumulation and other byproducts resulting from glycolysis [48,49]. Following this initial increase, the pH levels of all pomfret samples exhibited a gradual rise over time, eventually reaching a slightly alkaline or nearly neutral state. An upward trend was monitored in all experimentally contaminated samples after the first day, which signifies spoilage and the buildup of ammonia compounds [50]. While the pH levels increased at different rates, the groups treated with SAEW (G2, G3) demonstrated the slowest rate of increase. This rise in pH is linked to the gathering of many alkaline substances generated by endogenous enzymes and microorganisms present in the G1 and G4 samples. Consequently, the lowest pH recorded in the G2, G4, and G5 samples indicate considerable potential for preserving the pomfret's quality during cold storage.

The TBA value of the G1 increased rapidly to 2.93 mg/kg on the 3rd day of storage and the fish was spoiled by the 5th day. On the other hand, lower TBA values of 0.82, 0.73, 1.47, and 1.17 mg/kg were

obtained for G2, G3, G4, and G5, respectively ($p<0.05$). This shows a significantly stronger ability to restrain TBA increases ($p<0.05$), as mentioned by Xuan et al. [49]. Additionally, compared to the NEW treatment, the SAEW pretreatment kept the TBA value low at the end of storage ($p<0.05$). Fish quality is indirectly measured by TBA, which reflects lipid oxidation. According to Trigo et al. [51], lower TBA levels denote fresher fish, while larger values denote degradation. According to our findings, we can infer that electrolyzed water might slow down lipid oxidation. These results are in line with those of Luan et al. [52], who reported that using chitosan with electrolyzed water effectively extended the shelf life of hairtail and reduced the incidence of rancidity. Xuan et al. [49] discovered that SAEW-ice was excellent at preventing lipid oxidation, postponing spoiling, and getting rid of off flavors.

Total Volatile Basic Nitrogen (TVBN) serves as a significant metric for assessing the freshness of fish. It quantifies the occurrence of nitrogenous substances, including dimethylamine, ammonia, and trimethylamine, in fish sourced from both marine and freshwater environments [53]. Elevated levels of TVBN are indicative of the degradation of protein and non-protein nitrogen compounds, a process facilitated by bacterial activity and endogenous enzymatic reactions. These biochemical processes cause the creation of alkaline nitrogenous compounds, which can adversely affect the freshness and quality of the fish [54]. Although TVBN values can differ among fishery products, a threshold of 30 mg/100 g is generally regarded as the maximum acceptable level for fresh fish by consumers [48]. During storage at 4°C, TVBN amounts in all samples were observed to rise continuously. Notably, samples treated with electrolyzed water (EW), particularly those with strong alkaline electrolyzed water, exhibited a reduced rate of increase in TVBN, with strong alkaline electrolyzed water maintaining levels below the consumer-acceptable limit of 30 mg/100 g, as indicated by Wu et al. [48]. Furthermore, samples deemed unacceptable were identified on the ninth day in the group treated with new electrolyzed water (NEW) containing 0.5% NaCl.

Electrolyzed Oxidizing Water (EOW) is gaining recognition as a viable substitute for conventional sanitization techniques, such as heat treatment and chemical sanitizers. Slightly acidic electrolyzed water (SAEW) has demonstrated superior efficacy in increasing the shelf life of fish fillets when compared to neutral electrolyzed water (NEW). With a pH level between 2 and 5, SAEW is particularly potent against pathogens, leveraging its acidity to effectively manage microbial populations on surfaces. Conversely, NEW, which maintains a neutral pH, exhibits reduced effectiveness in microbial eradication relative to SAEW [55].

To elucidate the impact of SAEW and NEW on the virulence of *V. parahaemolyticus*, real-time PCR was utilized to assess the virulence of this bacterium through DNA quantification, owing to its notable sensitivity and specificity [56]. The findings indicate significant alterations between the control group and the treated groups, except G2, where the lowest expression levels for the tdh and toxR genes were recorded in G5. Regarding the transcription levels of the trh gene, all treated samples exhibited reduced expression compared to G1 samples ($p < 0.05$), with G3 demonstrating the lowest levels. These observations support with the results of Wang et al. [57], who indicated that real-time PCR data showed that AEW treatment can effectively inhibit the proliferation of *V. parahaemolyticus* cells in shrimp.

Conclusion and Recommendations

The results of our study show that using Slightly Acidic Electrolyzed Water (SAEW) and Neutral Electrolyzed Water (NEW) led to a significant decrease in the presence of *V. parahaemolyticus* in tilapia fish fillets. These treatments also helped maintain the microbiological, sensory, and physicochemical qualities of the fillets during storage. SAEW can prolong the shelf life of fillets by up to 4 days and reduce the expression of virulence genes. With a pH range of 5.5–6.5, SAEW is a promising nonthermal disinfection method and could reduce the need for free chlorine in disinfection processes. Future research should investigate its commercial applications and its effectiveness on other fish species and pathogens.

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Conflicts of interest

According to the authors, there isn't a conflict of interest.

Funding statement

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TABLE 1. Design for studying the effect of EW on experimentally contaminated fish fillets with *V. parahaemolyticus.*

Group	*V. parahaemolyticus*	SAEW	NEW	0.2% NaCl	0.5% NaCl
G0	-	-	-	-	-
G1	+	-	-	-	-
G2	+	+	-	+	-
G3	+	+	-	-	+
G4	+	-	+	+	-
G5	+	-	+	-	+

TABLE 2. Primers sequences, target genes, amplicon sizes, and cycling conditions.

Target gene	Primers sequences 5'-3'	Amplified segment (bp)	Amplification (35 cycles)				Final extension	References
			Primary denaturation	Secondary denaturation	Annealing	Extension		
Trh	GGCTCAAAATGGTTAAGCG CATTTCCGCTCTCATATGC	250	94˚C 5 min.	94˚C 30 sec.	54˚C 30 sec.	72˚C 30 sec.	72˚C 7 min.	[23]
tdh	CCATCTGTCCCTTTTCCTGC CCAAATACATTTTACTTGG	373	94˚C 5 min.	94˚C 30 sec.	54˚C 40 sec.	72˚C 40 sec.	72˚C 10 min.	[23]
toxR	GTC TTC TGA CGC AAT CGT TG ATA CGA GTG GTT GCT GTC ATG	368						[24]

TABLE 3. Primers sequences, target genes, amplicon sizes, and cycling conditions for SYBR green rt-PCR.

Target gene	Primers sequences	Amplification (40 cycles)					Reference
		Reverse transcription	Primary Denaturation	Secondary denaturation	Annealing (Optics on)	Extension	
16Sr RNA (housekeeping)	CAGGCCTAACACATGCAAGTC GCATCTGAGTGTCAGTATCTGTCC	50˚C 30 min.	94˚C 15 min.	94˚C 15 sec.	50˚C 30 sec.	72˚C 30 sec.	[26]
tdh	CATCTGTCCCTTTTCCTGC CCAAATACATTTTACTTGG						[23]
toxR	GTCTTCTGACGCAATCGTTG ATACGAGTGGTTGCTGTCATG						[27]
trh	TTGGCTTCGATATTTTCAGTATCT CATAACAAACATATGCCCATTTCC						[27]

TABLE 4. Effect of SAEW and NEW with 0.2% and 0.5% NaCl on the *V. parahaemolyticus* count (log10 CFU/g) intentionally contaminated fish filet samples (Mean ± SD, n=3).

	G1	G2	G3	G4	G5
2 min./ RT	5.28 ± 0.5^{a}	4.78 ± 0.1^{b}	4.67 ± 0.1^{b}	5.01 ± 0.2^{a}	4.87 ± 0.3^{b}
5 min./ RT	4.91 ± 0.07^{a}	3.99 ± 0.08^{b}	3.87 ± 0.09^{b}	4.89 ± 0.05^{a}	4.59 ± 0.5^{a}
10 min./ RT	4.82 ± 0.3^{a}	2.06 ± 0.05^{c}	1.76 ± 0.2^{c}	2.94 ± 0.05^{b}	2.67 ± 0.3^{b}
1st day/ 4°C	5.53 ± 0.2^{a}	1.59 ± 0.2^{c}	No growth	2.82 ± 0.1^{b}	2.33 ± 0.2^{b}
3rd day/ 4°C	spoiled	No growth	No growth	2.41 ± 0.4^{a}	2.01 ± 0.1^{a}
5th day/ 4°C	spoiled	1.81 ± 0.2^{b}	1.66 ± 0.1^{b}	3.43 ± 0.3^{a}	3.34 ± 0.3^{a}
7th day/ 4°C	spoiled	3.54 ± 0.04^{c}	3.23 ± 0.6^{c}	4.83 ± 0.2^{a}	4.13 ± 0.1^{b}
9th day/ 4°C	spoiled	4.58 ± 0.3^{a}	4.28 ± 0.5^{b}	spoiled	4.73 ± 0.3^{a}

Different superscript alphabetical letters within the same raw mean significant using ANOVA at *p-value* < 0.05.

TABLE 5. Effect of SAEW and NEW with 0.2% and 0.5% NaCl on pH of *V. parahaemolyticus* artificially contaminated fish fillet samples

Groups	G0	G1	G2	G3	G4	G5
1st day	6± 0.2^{a}	6.2± 0.2^{ab}	5.8± 0.1^{abc}	5.8± 0.2^{acd}	6.1± 0.1^{abe}	6.1± 0.2^{abc}
3rd day	6.2± 0.2^{a}	7.2± 0.1^{b}	6± 0.1^{ac}	5.9±0.3^{cd}	7± 0.2^{abd}	6.8± 0.2^{ab}
5th day	6.6± 0.1^{a}	spoiled	6.2± 0.1^{b}	6.2± 0.3^{abc}	7.1± 0.2^{d}	6.9± 0.1^{ac}
7th day	7± 0.2^{a}	spoiled	6.2± 0.2^{ab}	6.5± 0.4^{ab}	7.3± 0.2^{c}	6.9± 0.1^{a}
9th day	spoiled	spoiled	6.8± 0.2^{a}	6.9± 0.3^{a}	spoiled	7.3± 0.2^{a}

Different superscript alphabetical letters within the same raw mean significant using ANOVA at *p-value* < 0.05.

TABLE 6. Effect of SAEW and NEW with 0.2% and 0.5% NaCl on TBA level of *V. parahaemolyticus* artificially contaminated fish fillet samples

Groups	G0	G1	G2	G3	G4	G5
1st day	0.53 ± 0.02^{a}	1.43 ± 0.06^{b}	0.65 ± 0.03^{ac}	0.6 ± 0.02^{d}	1.1 ± 0.1^{e}	0.91 ± 0.02^{e}
3rd day	1.13 ± 0.06^{a}	2.93 ± 0.15^{b}	0.82 ± 0.02^{c}	0.73 ± 0.03^{d}	1.47 ± 0.06^{e}	1.17 ± 0.06^{f}
5th day	1.27 ± 0.06^{a}	spoiled	1.1 ± 0.1^{ab}	1.04 ± 0.1^{bc}	1.55 ± 0.005^{d}	1.29 ± 0.09^{abc}
7th day	1.63 ± 0.11^{a}	spoiled	1.6 ± 0.1^{ab}	1.2 ± 0.1^{bc}	2.23 ± 0.15^{ad}	1.92 ± 0.07^{ae}
9th day	spoiled	spoiled	1.68 ± 0.02^{a}	1.28 ± 0.07^{b}	spoiled	2.9 ± 0.17^{c}

Different superscript alphabetical letters within the same raw mean significant using ANOVA at *p-value* < 0.05.

TABLE 7. Effect of SAEW and NEW with 0.2% and 0.5% NaCl) on TVBN level of *V. parahaemolyticus* artificially contaminated fish fillet samples

Groups	G0	G1	G2	G3	G4	G5
1st day	14.3 ± 0.25^{a}	22.3 ± 0.64	15 ± 0.15^{ab}	14.6 ± 0.4^{b}	18.2 ± 0.36	17.6 ± 0.46
3rd day	22 ± 0.46	31.3 ± 1.53	15.5 ± 0.25^{c}	14.9 ± 0.12^{c}	20.6 ± 0.51^{e}	19.9 ± 0.32^{e}
5th day	24.5 ± 0.49	spoiled	17.8 ± 0.47^{b}	15.6 ± 0.4	21.2 ± 0.01	19.3 ± 0.66^{b}
7th day	30.5 ± 0.91	spoiled	21.6 ± 0.56^{b}	19.3 ± 0.66^{b}	28.5 ± 1.32	26.3 ± 1.53
9th day	spoiled	spoiled	29.3 ± 1.53	23.7 ± 0.58	spoiled	32.1 ± 1.25

Different superscript alphabetical letters within the same raw mean significant using ANOVA at *p-value* < 0.05.

TABLE 8. Effect of SAEW and NEW on the expression of virulence genes tdh, trh and toxR genes by using qRT-PCR

Items	G1	G2	G3	G4	G5
tdh	1.00±0.00^{a}	0.95±0.02^{a}	0.02±0.001^{c}	0.09±0.01^{b}	0.005±0.001^{c}
toxR	1.00±0.00^{a}	0.81±0.12^{a}	0.39±0.01^{b}	0.36±0.02^{b}	0.01±0.001^{c}
trh	1.00±0.00^{a}	0.50±0.06^{b}	0.02±0.002^{d}	0.21±0.02^{c}	0.15±0.01^{c}

Different superscript alphabetical letters within the same raw mean significant using ANOVA at *p-value* < 0.05.

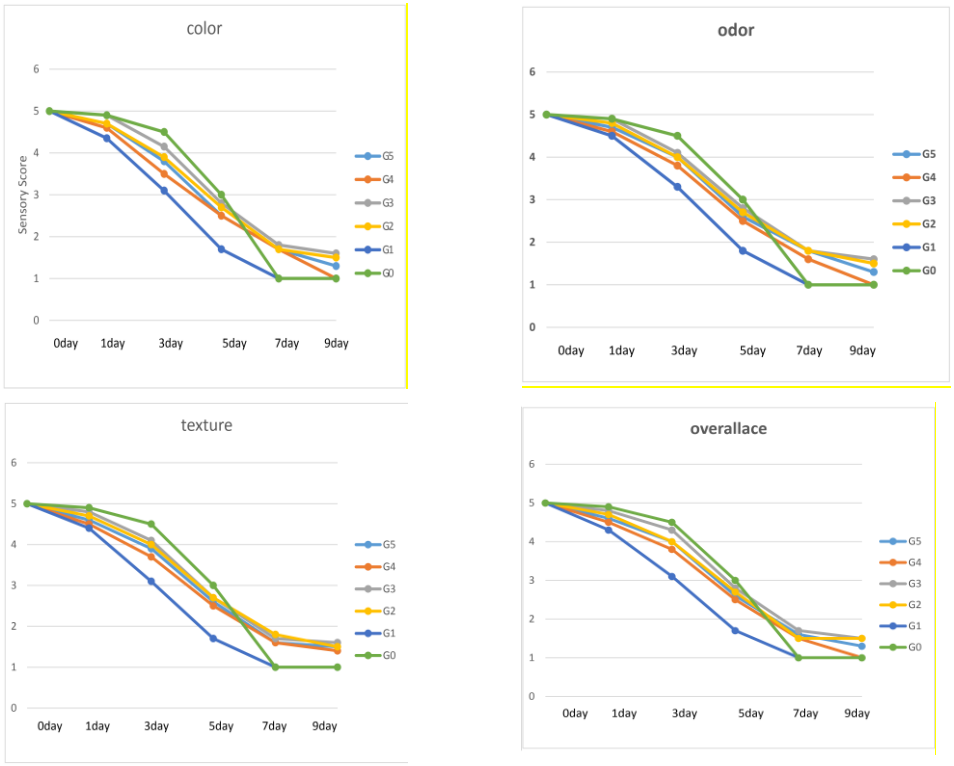

Fig. 1. Effect of SAEW and NEW with 0.2% and 0.5% NaCl on the Sensory characters of artificially contaminated fish fillet samples with V. parahaemolyticus during cold storage (color, odor, texture, and overall acceptability)

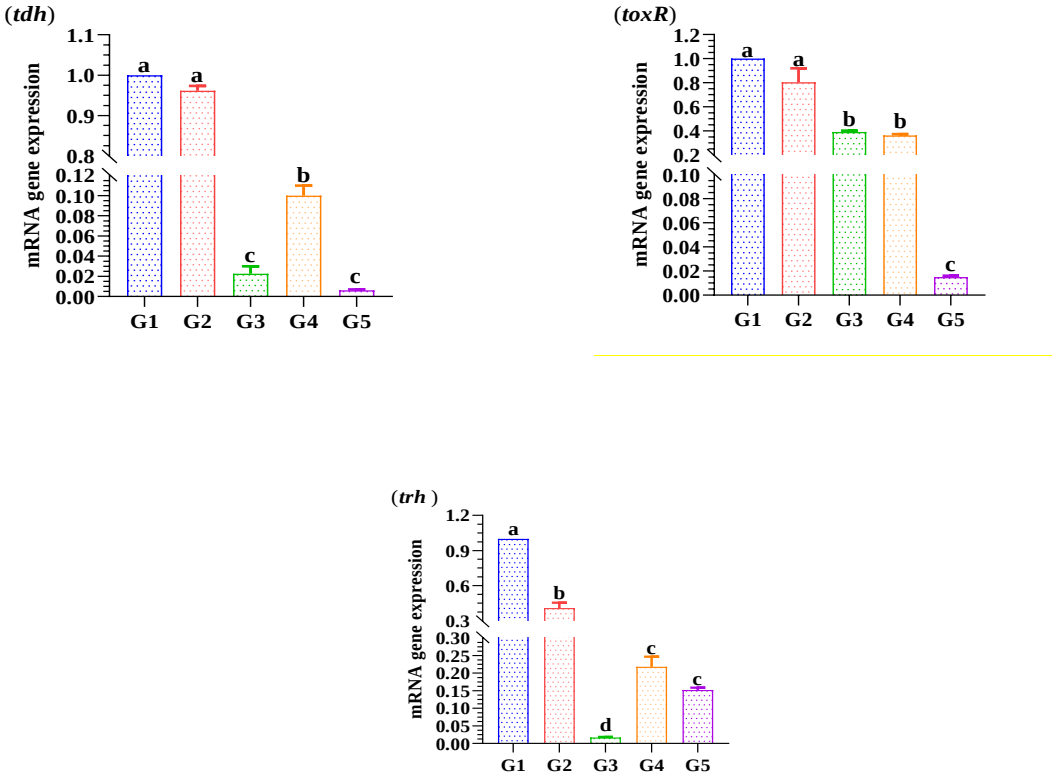

Fig. 2. Changes in the expression of *tdh*, *toxR* and *trh* genes as a response to treatments with SAEW and NEW.

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تقييم كفاءة المياه المحللة كهربائيا في السيطرة على تلوث شرائح السمك ببكتيريا *V. parahaemolyticus*

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الملخص

في الوقت الحاضر، يتم التعرف على المياه المحللة كهربائيًا (EW) على نطاق واسع كبديل للمضادات الحيوية الكيميائية لتقليل التلوث الميكروبي وإطالة العمر الافتراضي للغذاء. في هذا العمل، يتم استخدام شرائح سمك البلطي الملوثة عمدًا بـ *Vibrio parahaemolyticus* لفحص التأثيرات المضادة للبكتيريا للمياه المحللة كهربائيًا الحمضية قليلاً (SAEW) والمحايدة (NEW) بكميتين من كلوريد الصوديوم (٠,٢٪ و ٠,٥٪) على حدة. تدرس الدراسة أيضًا تأثيرها على جودة وخصائص أسماك البلطي الحسية والتعبير عن جينات الضراوة tdh و trh و toxR باستخدام qRT-PCR. تم إصابة عينات من شرائح سمك البلطي بشكل مصطنع بـ *V. parahaemolyticus*، ثم تم غمرها بشكل منفصل في SAEW و NEW (٠,٢٪ و ٠,٥٪ NaCl) لمدة ٢ و ٥ و ١٠ دقائق في درجة حرارة الغرفة، وبعد ذلك تم الاحتفاظ بالعينات في الثلاجة عند ٤ ± ١ درجة مئوية. أظهرت النتائج أن أعداد *V. parahaemolyticus* في اليوم الثالث من التخزين انخفضت مع NEW وتم تثبيطها تمامًا باستخدام SAEW. بالإضافة إلى ذلك، تم إطالة العمر الافتراضي لجميع عينات الشرائح المعالجة إلى اليوم السابع والتاسع من خلال إبطاء تدهور الرائحة واللون مقارنة بالعينات غير المعالجة، والتي أصبحت غير صالحة للاستهلاك. على وجه التحديد، أظهرت SAEW التي تحتوي على ٠,٥٪ NaCl خصائص فيزيائية وكيميائية أفضل. كان هناك أيضًا انخفاض كبير في التعبير عن جين الضراوة بين العينة غير المعالجة والعينات المعالجة الأخرى. وفي الختام، يمكن استخدام الماء المحلل كهربائيا كمعقم لتحسين الخصائص الميكروبية وزيادة مدة صلاحية شرائح السمك.

الكلمات الدالة: *V. parahaemolyticus*، شرائح سمك البلطي، الماء المحلل بالكهرباء، qRT-PCR، TBA، TVB-N، مدة الصلاحية.