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A Trial to Control *Bacillus cereus* on Soft Cheese Using Electrolyzed Water

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Abstract

Bacillus cereus is a frequent pathogenic contaminant of the Egyptian white soft cheese as the bacteria and its spores can withstand the industrial conditions of such products. With a growing search for sustainable, cheap and eco-friendly disinfection and sanitation technologies in food industry sector, Electrolyzed water with all its types are innovative and promising solutions that improve safety, quality, and operational efficiency of this field. In this research trial we studied the possibility of the electrolyzed water, either alkaline (AEW) or neutral (NEW) or combined, to control *Bacillus cereus* and act as a safe preservative to white soft cheese (which is a type of our traditional white cheese made from raw milk without any preservatives or even enough heat treatment as boiling or pasteurization of the used milk) . The experiment revealed a promising effect of both types (AEW & NEW) firstly on decreasing the number of *Bacillus cereus* bacteria in white soft cheese. Treatment with alkaline electrolyzed water for only 5 min reduced the count by 51.97%, while in using combined treatment of NEW followed by AEW, each for 5 min, the reduction percentage was 42.58%, meanwhile the highest reduction was observed in cheese treated with NEW alone for 5 min. with reduction 86.16% in its count. Not just that but both AEW and NEW showed considerable second effect on downregulation of the pathogenic gene expression of (dnaJ, hbL, cytK, Ent-FM, nhe-hbLD). So, we can conclude that neutral electrolyzed water has better effect on both reducing the *Bacillus ceruse* count and decline the pathogenic activity of its pathogenic genes.

Keywords: *Bacillus cereus*, Pathogenic genes, Food borne disease, Alkaline Electrolyzed water (AEW), Neutral electrolyzed water (NEW).

Introduction

Ensuring food safety is at the core of all research focused on food hygiene. Before any product reaches consumers, it must undergo thorough cleaning and sterilization to guarantee its safety. The use of chemical agents is common in this process; they are intended to suppress microbial activity, maintain product quality, and prolong shelf life. Yet, improper disposal of these chemicals can create significant challenges. Environmental pollution, contamination from chemical residues, and the risk of allergic reactions in consumers are all potential consequences. In essence, while these substances play a critical role in food preservation, their use and disposal require careful management to prevent unintended harm. [1].

In recent years, numerous advanced nonthermal sterilization methods have been developed in food processing. Techniques such as gamma irradiation, ultraviolet light treatment, ultrasound application, pulsed electric fields, cold plasma technology, high-pressure processing, and various forms of electrolyzed water have demonstrated significant potential for ensuring food safety without relying on conventional heat-based approaches. These innovations reflect the growing emphasis on preserving food quality while effectively eliminating microbial contaminants.[2].

Electrolyzed water has become a notable innovation in disinfection technology, especially in the food sector. Over the years, it has gained widespread acceptance to guarantee comprehensive sterilization of surfaces and maintain food safety standards. Its efficacy in these areas is well-established and has significantly enhanced hygiene practices. [3].

The idea of electrolyzed water was initially introduced in Russia within the agricultural sector [4]

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and has since expanded into various other domains, including global food safety. It has been extensively utilized for multiple applications, such as disinfection, water regeneration, and water decontamination in Japan since 1980. Over time, it has also been adopted in other critical sectors like the food industry, agriculture, livestock management, and clinical applications [5].

Electrolyzed water (EW) is generated in an electrolysis chamber by combining a solution of dilute sodium chloride (NaCl) with tap water, notably without incorporating any harmful chemical additives. [6]

This solution exhibits broad-spectrum antimicrobial properties, effectively targeting a range of microorganisms—such as bacteria, viruses, spores, fungi, and resilient biofilms—commonly present in chronic wounds and on environmental surfaces. [7].

Electrolyzed water (EW) is categorized into different types according to its pH level. These categories include strongly acidic EW (pH 2.2–2.7), weakly acidic EW (pH 2.7–5.0), slightly acidic EW (pH 5.0–6.5), neutral EW (pH 6.5–7.5), and alkaline EW (pH 11.0–13.8). Each category represents a unique point on the pH scale, from highly acidic to strongly alkaline. [8].

In recent years, there has been a growing interest in the creation of both acidic electrolyzed water (AEW) and neutral electrolyzed water (NEW) as sanitizing solutions. These products are produced by applying electrolysis to a diluted sodium chloride (NaCl) solution, specifically by passing it through the anode compartment of a membrane electrolyzer. [9]. The use of electrolyzed water in various industries has shown its effectiveness as a safe and economical sanitizer in sectors such as food, aquaculture, agriculture, medical, and energy. Recently, many start-up companies and industries have started to commercialize and promote different types of electrolyzed water worldwide, making it accessible to all these sectors. [10]

The EPA specifically recommended using disinfectants containing hypochlorite acid as the active ingredient for surface disinfection against COVID-19. This reflects the agency's position that such compounds are effective in reducing viral contamination on commonly touched surfaces.[11].

Bacillus cereus is a spore-forming, Gram-positive, rod-shaped bacterium that exhibits motility. It can grow in both aerobic and facultative conditions. This organism is widespread in various environments and is recognized as a significant human pathogen, capable of causing both emetic (vomiting) and diarrheal illnesses. [12].

B. cereus is commonly present in environments such as dust, soil, and even culinary spices. Remarkably, it can withstand typical cooking processes by forming heat-resistant spores. If cooked food is subsequently stored at improper temperatures, these spores may germinate, leading to substantial bacterial proliferation.. [13].

Emetic syndrome occurs after ingesting food that has been tainted with a pre-formed toxin called cereulide (also known as emetic toxin). The production of cereulide is regulated by the ces gene cluster, which is made up of seven coding DNA sequences: cesH, cesP, cesT, cesA, cesB, cesC, and cesD. It is important to note that these genes are situated on a plasmid.[14].

Three primary enterotoxins are implicated in the diarrheal syndrome: hemolysin BL (HBL), nonhemolytic enterotoxin (NHE), and cytotoxin K (CytK).

The genes responsible for encoding HBL are located within the hbl operon, which consists of hblC, hblD, and hblA. The nhe operon encodes NHE and is made up of the nheA, nheB, and nheC genes. The cytK gene is responsible for encoding CytK. Importantly, all of these genes are situated on the bacterial chromosome. [15].

Egyptian cheeses are considered an important part of the Egyptian diet. This cheese is most often produced using buffalo or cow milk, though it's not uncommon to encounter versions made from sheep, goat, or even camel milk. It holds the distinction of being Egypt's most prevalent cheese The cheese then matures in a highly salted whey brine at room temperature, typically for a period of four to six months, [16].

As outlined in the Egyptian standard [17] soft cheese must comply with specific microbiological criteria. It should not contain any pathogenic microorganisms or their toxins, or visible fungal growth. Additionally, the presence of *L.monocytogenes* and *E. coli* is strictly prohibited.

The acceptable limits are as follows: coliform counts must not exceed 10 cfu/g, total mold counts should be no greater than 10 cells/g, and total yeast counts must remain at or below 400 cells/g. These standards are established to ensure the safety and quality of soft cheese products.

Food-borne bacteria have long dominated research and surveillance efforts in the realm of gastrointestinal diseases. *Bacillus* species, in particular, are infamous for their tough endospores, which present ongoing headaches for food producers trying to keep their products safe. [8]

The primary objective of this study was to evaluate the efficacy of alkaline electrolyzed water (AEW) and neutral electrolyzed water (NEW) in reducing populations of the pathogen *Bacillus cereus*. White soft cheese, a widely consumed

traditional Egyptian food, often harbours this biological hazard, posing a risk to consumers. To gain further insight, the researchers employed PCR techniques to examine gene expression changes in Bacillus cereus strains isolated from cheese samples following treatment.

Material and Methods

Refreshment and Enumeration of Bacillus cereus Reference Strain

The *B. cereus* reference strain (NCTC 7464/ ATCC 10876, batch 2001-2025) was revitalized and quantified following the method described by Rasool et al. [18].

A single, typical colony was inoculated into 10 ml of nutrient broth and incubated overnight to establish a fresh broth culture. This culture was then used as the source for re-cultivation, allowing for the isolation of pure colonies. These purified colonies were subsequently submitted for PCR analysis to identify the presence of specific virulence genes in the strain.

PCR for Detection of Virulence Genes in Bacillus cereus

The procedure generally adhered to the approach described by Ehling-Schulz et al. [19], with minor modifications as noted below.

A. DNA Extraction

Genomic DNA was isolated using the QIAamp DNA Mini Kit (Qiagen, Germany, GmbH), with minor modifications to the manufacturer's protocol. In summary, 200 µl of the sample suspension was combined with 10 µl of proteinase K and 200 µl of lysis buffer, then incubated at 56°C for 10 minutes. Subsequently, 200 µl of absolute ethanol was added. The samples were processed through the recommended washing and centrifugation steps. Finally, DNA was eluted using 100 µl of elution buffer.

B- Oligonucleotide Primers

Primers were sourced from Metabion (Germany), and their specific sequences are detailed in Table 1.

C. PCR Amplification

The amplification protocol is further described below. For each PCR reaction, a total volume of 25 µl was prepared, containing 12.5 µl EmeraldAmp Max PCR Master Mix (Takara, Japan), 1 µl of each primer (20 pmol), 5.5 µl sterile water, and 5 µl of DNA template. Amplifications were performed using an Applied Biosystems 2720 thermal cycler.

D. Analysis of PCR Products

PCR products were separated by electrophoresis on 1% agarose gel (Applichem, Germany, GmbH) in 1X TBE buffer at room temperature using a voltage gradient of 5 V/cm. Twenty microliters of PCR product was loaded into each gel slot. Molecular size markers used were Generuler 100 bp ladder (Fermentas, Germany) and GelPilot 100 bp plus ladder (Qiagen, GmbH). Gel images were captured with an Alpha Innotech gel documentation system and analyzed using dedicated software.

Electrolyzed Water Device and experimental work:

A. Electrolyzed water Device

An electrolyzed water device was constructed in the laboratory to generate both alkaline and neutral electrolyzed water for experimental use. Simple apparatus consisting of two chambers each one containing one litre of tab water with 2 gm. NaCl salt for each litre of water, and in this water an electric current was used from 400 wat adaptor to electrically analyses this solution.

B. Preparation of Overnight broth culture and its serial dilution

The *B. cereus* reference strain (NCTC 7464/ATCC 10876, batch 2001-2025) was cultured on Bacillus cereus Agar Base (PEMBA) at 30–32°C for 24 hours. After incubation, characteristic peacock blue colonies, measuring approximately 3–5 mm with a surrounding halo, were selected and transferred into sterile nutrient broth tubes containing 10 ml of broth. These tubes were incubated at 37°C for an additional 24 hours.

Following incubation, the broth culture was serially diluted to a 10^{-3} dilution. This dilution was used in the experiment and yielded a bacterial count ranging from 3×10^2 to 6×10^2 cfu across three repeated trials.

C. The experimental work

For the experimental setup, four separate groups of cheese samples were prepared. Each group included 30 grams of white soft cheese, which was purchased from a supermarket in sterile packaging and immediately transported to the laboratory in an ice box to maintain freshness. The 30 grams of cheese from each group were divided into three cups, with each cup containing 10 grams of cheese and 10 milliliters of whey. To each cup, 1 milliliter of an overnight B. cereus broth was aseptically inoculated.

The mixtures were left to incubate for approximately two hours to ensure sufficient inoculation. Following this period, the next experimental steps involved the addition of various types of electrolyzed water.

Each group; included three cups (100ml plastic disposal serial cup) each cup containing 10 grams of soft cheese and 10 ml. of whey (as whey makes it easy for the 1ml broth with *B.cereus* bacteria to be evenly distributed on the cheese). The groups were treated as follows:

Group A: control positive cheese group; inoculated with *B. cereus* and received no treatment.

Group B: inoculated with *B. cereus* and treated with 10 ml of alkaline electrolyzed water for 5 minutes.

Group C: inoculated with *B. cereus* and first received 10 ml of alkaline electrolyzed water for 5 minutes, immediately followed by 10 ml of neutral electrolyzed water for another 5 minutes.

Group D: inoculated with *B. cereus* and treated with 10 ml of neutral electrolyzed water for 5 minutes.

This experimental protocol was repeated three times, resulting in a total of 36 cheese samples subjected for analysis for enumeration of *B. cereus* using *B. cereus* agar plates according to [18].

PCR for Gene Expression Analysis of RNA from Different Treatments Compared to Original Strain

A. RNA Extraction

To preserve RNA integrity, 0.5 ml of harvested bacterial culture was combined with 1 ml of RNAprotect Bacteria Reagent (Qiagen, Germany), vortexed briefly, and incubated at room temperature for 5 minutes. The samples were then centrifuged at 8000 rpm for 10 minutes, after which the supernatant was discarded. The resulting pellet was resuspended in 200 µl TE buffer containing 1 mg/ml lysozyme (Biochemica, Applichem). Subsequently, 700 µl RLT buffer supplemented with 10 µl β-mercaptoethanol per ml was added, followed by 500 µl of 100% ethanol. RNA extraction was carried out using the QIAamp RNeasy Mini Kit, including an on-column DNase digestion step.

B. Oligonucleotide Primers

Primers supplied by Metabion (Germany) were used (listed in Table 1).

C. SYBRGreenReal-TimePCR

The PCR reactions were prepared with 12.5 µl of 2X QuantiTect SYBR Green PCR Master Mix (Qiagen), 0.25 µl RevertAid Reverse Transcriptase (200 U/µL, Thermo Fisher), 0.5 µl primers (20 pmol), 8.25 µl nuclease-free water, and 3 µl RNA template. Amplification was conducted using a Stratagene MX3005P real-time PCR system.

D. DataAnalysis

Genomic DNA was isolated using the QIAamp DNA Mini Kit (Qiagen, Germany), incorporating slight adjustments to the manufacturer's protocol. Briefly, 200 µl of the sample suspension was mixed with 10 µl of proteinase K and 200 µl of lysis buffer, followed by incubation at 56°C for 10 minutes. Next, 200 µl of absolute ethanol was added. The mixture then underwent the recommended washing and centrifugation steps. Finally, DNA was eluted in 100 µl of elution buffer.

Statistical Analysis

Statistical analyses were conducted using IBM SPSS version 20. Normality of the data was assessed via the Shapiro-Wilk test. Quantitative variables are reported as range (minimum–maximum), mean, and standard deviation. For comparisons between two groups, Student's t-test was utilized. When comparing four groups, one-way ANOVA was applied, followed by Tukey's post hoc test for pairwise comparisons. Statistical significance was considered at a P-value less than 0.05.

Results

In the PCR analysis of the *Bacillus cereus* reference strain, multiple pathogenic genes were detected, including hbl, cytK, nhe, hblD, and Ent-FM; notably, the ces gene was not observed (see Table 2 for details).

Regarding the cheese samples, the control group, the untreated group (Group I) exhibited a mean *Bacillus cereus* count of $4.26 \times 10^2 \pm 1.14 \times 10^2$ CFU, with individual values ranging from 3×10^2 to 6×10^2 CFU. Application of AEW treatment for five minutes (Group II) resulted in a marked reduction, yielding a mean count of $2.04 \times 10^2 \pm 9.5 \times 10$ CFU (range: 0–3×10^2 CFU), corresponding to a 51.97% decrease. The sequential treatment involving AEW for five minutes followed by NEW for an additional five minutes (Group III) produced a mean count of $2.44 \times 10^2 \pm 1.35 \times 10^2$ CFU (range: 4x10–4.5×10^2 CFU), equating to a 42.58% reduction. The most substantial decline was achieved with NEW treatment alone for five minutes (Group IV), which produced a mean count of $5.89 \times 10^2 \pm 9.5 \times 10$ CFU (range: 0–2.3×10^2 CFU), reflecting an 86.16% reduction (refer to Table 3).

Figure 2 illustrates the percentage reduction observed in groups II, III, and IV following their respective treatments. Specifically, the groups underwent five minutes of AEW exposure, with some groups receiving an additional five minutes of NEW treatment were posed reduction percent of 51.97% and 42.58%, respectively. Notably, group IV, which was treated with five minutes of NEW, exhibited the highest reduction percentage among all groups.

Gene expression analysis using SYBR Green RT-PCR demonstrated a marked reduction in virulence gene expression in treated samples compared to controls. Specifically, fold changes for hblD, nhe, Ent-FM, cytK, and hbl ranged from 0.06 to 0.81, depending on the treatment group. Notably, the 5-minute treatment with NEW resulted in the most pronounced decrease in gene expression (see Table 4). These findings underscore the significant inhibitory effect of EW, particularly NEW, on decreasing the pathogenesity expression of virulence genes.

Meanwhile Fig. 3. show Amplification curves of SYBR Green RT-PCR for *Bacillus cereus* (A) Ent-FM, (B) dnaJ gene, (C) cytK, (D) nhe, and (E) hbI, showing variable CT values for control and three diferant treated samples. As in table (4)

Discussion

Egyptian white soft cheese is produced mainly from raw milk which constitute the main sources of bacterial contamination including spore-forming pathogens as *Bacillus cereus*, in addition to the environmental conditions of cheese manufacturing.

Hassan and Gomaa (2016) identified *B. cereus* in 18%, and 10% of the fresh soft Domiati cheese samples from Cairo and Giza Governorates, respectively [20]

Electrolyzed water has recently attracted considerable attention in the context of food sanitation and the management of plant pathogens. Its applications are diverse, ranging from seed treatment and postharvest disease control to the suppression of fungal growth and mitigation of foodborne pathogens. Researchers have explored various forms of electrolyzed water—including acidic, alkaline, and neutral solutions—demonstrating broad interest in evaluating their effectiveness across multiple domains related to food safety and plant health. [26]

Electrolysis is conducted in specially designed equipment that keeps the cathode and anode chambers separate. At the cathode, hydrogen gas and hydroxide ions are produced, leading to the formation of an alkaline solution containing sodium hydroxide. At the anode, chloride ions are oxidized to produce chlorine gas. This chlorine is present in an acidic environment, which is particularly corrosive to metals. [12]

The chemical environment at the anode directly influences the products formed. In acidic solutions, chlorine gas is generated, whereas alkaline conditions favor the production of sodium hydroxide. Notably, producing an effective disinfectant, such as hypochlorous acid, requires maintaining the solution near a neutral pH. Otherwise, the desired sanitizing agent is not formed, and less effective or unintended products are produced. [21]

Hypochlorous acid is recognized as a weak acid and a notable oxidizing agent. [24]

In the context of our experiment, the highest efficacy was observed when the soft cheese was treated with NEW for a duration of only five minutes.

These results may be due to; at a pH of 7.3—slightly above neutral—the solution contains nearly equal amounts of hypochlorous acid and hypochlorite ion. When the pH decreases, the equilibrium shifts in favor of hypochlorous acid, which enhances the solution's disinfecting power. While both sodium hydroxide and hypochlorous acid functions as effective disinfectants, hypochlorous acid is most efficient under neutral to mildly acidic conditions, where it predominates and maximizes antimicrobial activity.[22]

Alkaline Electrolyzed Water (AEW) demonstrates notable efficacy as a sanitizing agent, particularly in its ability to clean surfaces and inhibit the development of biofilms. Its reputation for both effectiveness and safety underpin its widespread use as a sanitizer and disinfectant. Nevertheless, AEW does present certain limitations, most notably with regard to its storage stability. The active properties of these solutions diminish over time, necessitating the implementation of on-site electrolysis systems—often referred to as chlorine generators—especially in industrial, institutional, and municipal settings, to ensure a consistent supply of freshly generated AEW for optimal performance.[23]

These units eliminate the need to store or transport chlorine and avoid the logistical challenges of shipping prepared chlorine solutions. As of March 2016, cost-effective electrolysis units have also become available for residential and small business use.

Acidic electrolyzed water (AEW) and neutral electrolyzed water (NEW) have recently gained attention as effective sanitizing agents. Both are produced by electrolyzing a dilute sodium chloride (NaCl) solution in the anode compartment of a membrane electrolyzer. This process generates solutions with notable antimicrobial properties, making them useful in various sanitation applications. [25]

Bacillus cereus is an incredibly resilient, spore-forming bacterium found widely in the environment, including soil and food sources. Notably, it is recognized as a significant biological hazard due to its capacity to cause foodborne illnesses—most famously, the emetic and diarrheal syndromes commonly associated with contaminated foods. The emetic (vomiting) syndrome emerges following the consumption of food that already contains preformed cereulide toxin. [14].

The production of this toxin results from the activity of the ces gene cluster, which includes seven genes: cesH, cesP, cesT, cesA, cesB, cesC, and cesD. Notably, this gene cluster is located on a plasmid rather than on the bacterial chromosome [29].

Beyond its role in food poisoning, B. cereus can also be responsible for a range of local and systemic infections. In severe cases, these infections may result in significant health complications and, according to reports, can lead to death in approximately 10% of affected individuals. [28]

Foodborne illnesses linked to B. cereus group strains do not require antibiotic treatment. However, B. cereus bacteraemia, which can result in severe and life-threatening systemic infections, particularly in immunocompromised individuals, necessitates the use of suitable antibiotic therapies. The widespread use of antimicrobials can contribute to the development of antimicrobial-resistant strains (AMRs), potentially leading to the ineffectiveness of standard treatments. Therefore, ensuring food safety and the absence of this pathogenic microorganism is significantly preferable to treatment. [31-30]

In this investigation, we assessed the disinfectant properties of two varieties of electrolyzed water: neutral electrolyzed water (with a pH between 6.5 and 7.5) and alkaline electrolyzed water (pH ranging from 11.0 to 13.8). The study focused on their effectiveness against *Bacillus cereus* (reference strain TCS, 2001-2025) inoculated in the white soft cheese. Notably, this cheese is commonly produced without undergoing heat treatment, or adding any preservatives rendering it particularly vulnerable to contamination by foodborne pathogens.

Incorporating a straightforward, cost-effective, and efficient measure—specifically, applying neutral electrolyzed water after cheese production and prior to storage—offers clear benefits. Electrolyzed water, in its various forms, is increasingly recognized as an innovative sanitizer capable of effectively reducing bacterial contamination on both foods and processing equipment [35]. This step not only enhances the overall safety of the product but also significantly lowers biological risks associated with cheese production.

The *Bacillus cereus* strain under investigation possesses several pathogenic genes, including Hbl, cytK, nhe, hblD, and Ent-FM, as confirmed by PCR analysis (see Table 1). Following treatment with NEW and AEW, further PCR assays (Table 3) were conducted to assess changes in the expression of these virulence genes. Both NEW and AEW were found to downregulate the expression of the targeted genes, as determined by SYBR Green RT-PCR. The fold changes for hblD, nhe, Ent-FM, cytK, and hbl ranged from 0.06 to 0.81, depending on the treatment group. Notably, treatments involving both the combination and the neutral electrolyzed water demonstrated greater reductions in gene expression. This downregulation of virulence genes is significant, as it may reduce the pathogenic potential of *Bacillus cereus*, supplementing the primary effect of decreasing bacterial counts.

The experimental setup consisted of four groups, each with nine samples, making a total of 36. Group I acted as the untreated control. Group II was exposed to alkaline electrolyzed water (AEW, pH 10–12) for five minutes. For Group III, samples underwent a sequential treatment: first AEW for five minutes, then neutral electrolyzed water (NEW) for another five minutes. Group IV received only NEW for five minutes.

Results demonstrated that both AEW and NEW significantly reduced Bacillus cereus counts and suppressed the expression of virulence genes. These findings are detailed in Tables 2 and 3.

Numerous studies align with these observations. For example, other study demonstrated that NEW exhibits bactericidal effects against robust endospore-forming bacteria such as Bacillus cereus and Clostridium perfringens on both fresh produce and polypropylene cutting boards at ambient temperature. [32]

Their findings indicate that NEW may serve as a practical alternative to conventional chemical sanitizers. Similarly, other researchers found that NEW achieved bacterial reduction on food preparation surfaces comparable to sodium hypochlorite, thus supporting its potential as an effective and safer disinfectant [33].

In summary, both neutral and alkaline electrolyzed water exhibit significant efficacy in reducing both the bacterial load and virulence gene expression of *Bacillus cereus* on white soft cheese, highlighting their promise as alternative disinfectants in food safety applications.

Conclusion

Research consistently demonstrates that Electrolyzed water (EW) has antimicrobial efficacy against a wide spectrum of pathogens, including both bacteria and viruses. Following its classification as "generally recognized as safe" by the United States Department of Agriculture (USDA), EW has achieved broad acceptance internationally and is now routinely employed as a sanitizer across the food industry. [36].

Currently, a variety of commercially available neutral electrolyzed water products exist. For instance, Aquaox™ Disinfectant 275 (Aquaox Industries Inc., Fontana, CA) is widely used. The primary active component in this product is hypochlorous acid at a concentration of 0.0275% (equivalent to 275 mg/L free available chlorine). This compound is generated through the electrochemical electrolysis of a diluted sodium chloride solution under neutral pH conditions. The resulting sanitizer is suitable for direct use in food safety applications [34].

We could conclude that; neutral electrolyzed water has demonstrated efficacy as a bactericidal treatment for ready-to-eat foods such as soft white cheese. Applying EW as a final surface treatment prior to retail distribution enhances product safety and helps reduce the risk of foodborne illness. So, it's preferable to use the step of adding EW for only

five min. before its storage to insure its freedom from many biological hazards as *Bacillus cereus*.

Therefore, Electrolyzed water (Neutral or, Alkaline) may have strong potential to help ensure food safety.

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Not applicable.

Availability of data and materials

Data and materials are available on reasonable request from the author.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

This research exclusively involved the analysis of food samples and bacterial cultures, without the use of human participants or live animals. Accordingly, formal ethical approval was not required in accordance with institutional and national guidelines for research ethics.

TABLE 1. Primers sequences, target genes, amplicon sizes and cycling conditions.

Target gene	Primers sequences	Amplified segment (bp)	Primary denaturation	Amplification (35 cycles)			Final extension	Reference
				Secondary denaturation	Annealing	Extension		
hbl	GTA AAT TAI GAT GAI CAA TTTC AGA ATA GGC ATT CAT AGA TT	1091	94˚C 5 min.	94˚C 30 sec.	49˚C 40 sec.	72˚C 1 min.	72˚C 10 min.	[19]
nhe	AAG CIG CTC TTC GIA TTC ITI GTT GAA ATA AGC TGT GG	766	94˚C 5 min.	94˚C 30 sec.	49˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	
cytK	ACA GAT ATC GGI CAA AAT GC CAA GTI ACT TGA CCI GTT GC	421	94˚C 5 min.	94˚C 30 sec.	49˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	
ces	GGTGACACATTATCATATAAGGTG GTAAGCGAACCTGTCTGTAACAACA	1271	94˚C 5 min.	94˚C 30 sec.	49˚C 40 sec.	72˚C 1.2 min.	72˚C 10 min.	
hblD	AGT TAT TGC AGC TAT TGG AGG GTC CAT ATG CTT AGA TGC TGT GA	148	94˚C 5 min.	94˚C 30 sec.	56˚C 30 sec.	72˚C 30 sec.	72˚C 7 min.	[38]
entFM	ATGAAAAAAGTAATTTGCAGG TTAGTATGCTTTTGTGTAACC	1269	94˚C 5 min.	94˚C 30 sec.	52˚C 40 sec.	72˚C 1.2 min.	72˚C 10 min.	[39]

TABLE 2. PCR results for the *Bacillus cereus* reference strain to determine its pathogenic genes.

Sample	Hbl	cytK	nhe	Ces	hblD	Ent-FM
B. cereus **(TCS,2001-2025)**	+	+	+	-	+	+

TABLE 3. Data analysis for comparison between the different studied groups of cheese treated with electrolyzed water (Total 36 sample) in different 4 groups.

	Group I (no treatment) (n = 9)	Group II (5min. with AEW) (n = 9)	Group III (5min. with AEW then 5 min. NEW) (n = 9)	Group IV (5 min. with NEW) (n = 9)	F	p
Min. – Max.	$3x10^2$-$6x10^2$	$0 – 3x10^2$	$4x10 – 4.5x10^2$	$0 – 2.3x10^2$		
Mean ± SD.	$4.26x10^2 \pm 1.14x10^2$	$2.04x10^2 \pm 9.5x10$	$2.44x10^2 \pm 1.35x10^2$	$5.89x10^2 \pm 9.5x10$	16.619*	<0.001*
p_0		0.001*	0.008*	<0.001*		
Sig. bet. Grps.		p_1=0.870,	p_2=0.043*,	p_3=0.006*		
% of reduction		51.97	42.58	86.16		

SD stands for standard deviation. The "F" refers to the F-statistic from the one-way ANOVA test, which assesses whether there are significant differences among the groups. Pairwise comparisons between each set of two groups were conducted using Tukey's Post Hoc Test. The lowercase "p" indicates the p-value for overall group comparisons. More specifically, p0 represents the p-value for comparing Group I with each of the other groups; p1 is for the comparison between Groups II and III; p2 compares Groups II and IV; and p3 compares Groups III and IV. An asterisk (*) denotes statistical significance at $p \leq 0.05$. AEW refers to alkaline electrolyzed water, and NEW stands for neutral electrolyzed water.

TABLE 4. CT values and relative expression fold changes of hblD, nhe, Ent-FM, and cytK genes in *Bacillus cereus* after treatment, compared to the control group.

Group	B. cereus Sample No	dnaJ	hbl		cytK		Ent-FM		nhe		hblD	
		CT	Fold change	CT	Fold change	CT	Fold change	CT	Fold change	CT	Fold change	CT
Control	B1	20.25	-	23.54	-	20.98	-	23.18	-	21.91	-	22.37
Treatment AEW For 5 min.	B2	20.44	0.2238	25.89	0.5359	22.07	0.4147	24.64	0.3143	23.77	0.4090	23.85
Treatment AEW For 5 min. then Treatment NEW for 5 min.	B3	20.80	0.0571	28.22	0.3322	23.12	0.0974	27.09	0.1303	25.40	0.2192	25.11
Treatment NEW For 5 min.	B4	20.19	0.4061	24.78	0.8066	21.23	0.6156	23.82	0.5434	22.73	0.7684	22.69

AEW: Alkaline Electrolyzed Water. NEW: Neutral Electrolyzed Water. CT: Cycle Threshold. hblD: Hemolysin BL D subunit gene. nhe: Non-hemolytic Enterotoxin gene. Ent-FM: Enterotoxin FM gene. cytK: Cytotoxin K gene. hbl: Hemolysin BL gene. dnaJ: Heat shock protein gene. B. cereus: *Bacillus cereus*.

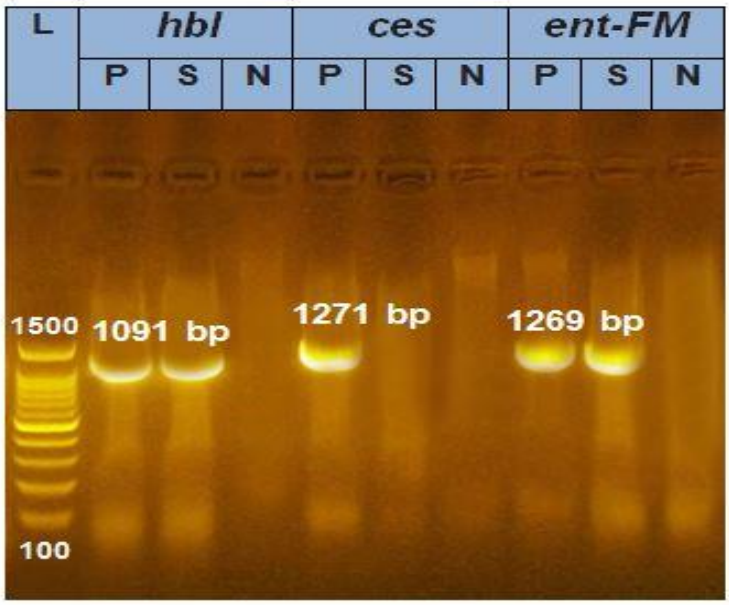

Fig. 1. Different pathogenic genes found in *Bacillus cereus* strain by PCR as in table (1)

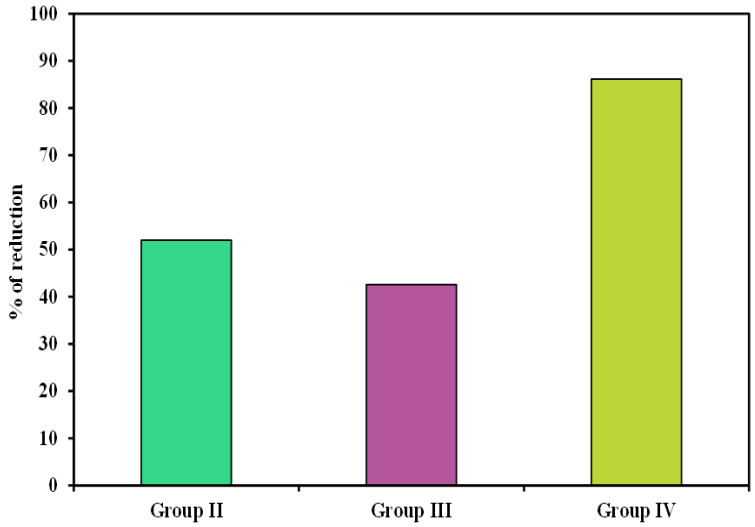

Fig. 2. Comparison between the three studied groups according to percentage of reduction in *Bacillus ceruse* count in the three treated different groups (II, III, IV) as group (I) was a control.

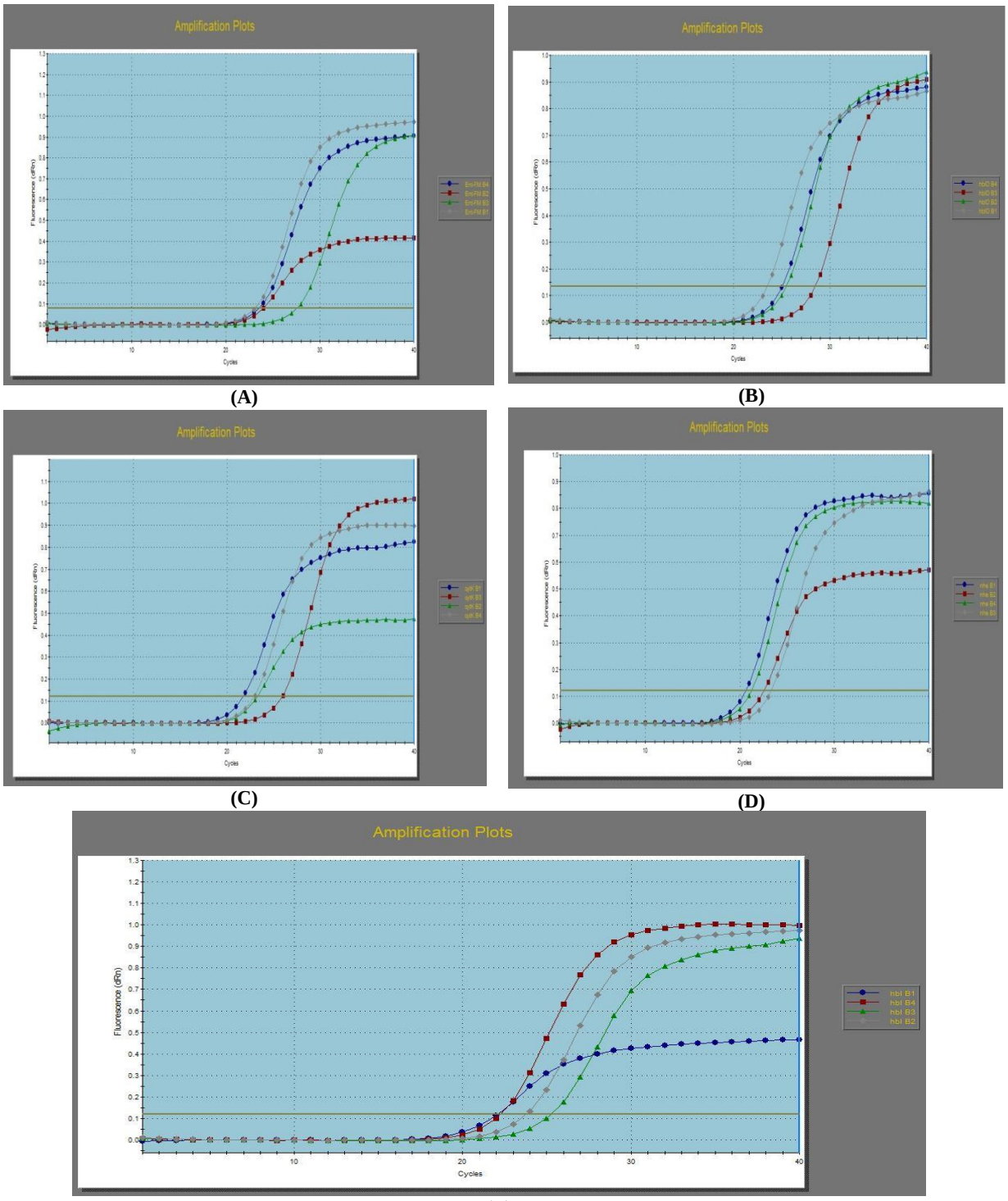

Fig. 3. Amplification curves of SYBR Green RT-PCR for *Bacillus cereus* **(A) Ent-FM, (B) dnaJ gene, (C) cytK, (D) nhe, and (E) hbI, showing variable CT values for control and treated samples. As in table (4)**

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تجربة للسيطرة على تواجد بكتيريا باسيليس سيرس في الجبن الطري باستخدام الماء المحلل كهربيا

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الملخص

في الوقت الحالي، تتجه الأنظار عالميًا نحو تبنّي تقنيات مستدامة وصديقة للبيئة، لا سيما في قطاع الأغذية الذي يسعى باستمرار لتطوير حلول مبتكرة تضمن السلامة والجودة والكفاءة في المنتجات الغذائية. من بين الابتكارات التي برزت مؤخرًا، يبرز استخدام الماء المعالج كهربائيًا – سواء كان قلويًا أو متعادلًا – والذي يُنتج عبر عملية التفكك الكهروكيميائي لمحلول ملحي (٢ جرام ملح لكل لتر ماء). هذا النوع من الماء يُعد خيارًا واعدًا، إذ أظهر فعالية في الحد من التلوث الميكروبي ومنع تكوّن البيوفيلم على الأسطح الملامسة للأغذية، مع الحفاظ على معايير عالية من النظافة والسلامة. تجدر الإشارة إلى أن بكتيريا باسيليس سيرس تُعد من أهم مسببات الأمراض المنقولة عبر الأغذية، نظرًا لقدرتها على تكوين أبواغ شديدة المقاومة، بالإضافة إلى حويصلات وسُموم تقاوم الحرارة، ما يجعلها قادرة على البقاء في الأغذية حتى بعد الطهي أو التخزين، كما هو الحال في بعض أنواع الجبن الأبيض. هذه السُموم قد تبقى نشطة في الأغذية النيئة والمطبوخة على حد سواء، ما يؤدي إلى حدوث حالات تسمم غذائي تظهر غالبًا في صورة متلازمة القيء و/أو الإسهال. في هذا البحث، تم تقييم تأثير الماء المعالج كهربائيًا – بنوعيه القلوي والمتعادل – كمادة حافظة للجبن الأبيض الطري. أظهرت النتائج أن كلا النوعين أسهما بوضوح في تقليل أعداد بكتيريا باسيليس سيرس في الجبن. كما لوحظ وجود تأثير على التعبير الجيني لبعض الجينات المرتبطة بقدرة البكتيريا على إحداث الأمراض، وكان الماء المعالج كهربائيًا المتعادل أكثر فعالية في تقليل أعداد هذه البكتيريا. استنادًا إلى النتائج، يمكن اعتبار الماء المعالج كهربائيًا وسيلة فعّالة وصديقة للبيئة لتعزيز سلامة المنتجات الغذائية، خصوصًا في صناعة الأجبان الطرية، مع المحافظة على الجوانب البيئية والصحي.

تشير نتائج هذا البحث إلى إمكانية التوصية باستخدام الماء المحلل كهربياً المتعادل، بحيث يُضاف إلى الجبن بعد اكتمال عملية التصنيع وقبل التخزين. بما أن هذا الماء متعادل كهربياً، فهو لا يؤثر على الصفات الظاهرية للجبن، كما لوحظ أن له تأثيراً إيجابياً في تقليل أعداد البكتيريا والفطريات التي قد تتواجد في المنتج. ومن الجدير بالذكر أن هذا الماء غير قابل للتخزين لفترات طويلة؛ إذ يفقد خصائصه الكهربائية تدريجياً ويعود إلى كونه محلولاً ملحياً عادياً خلال فترة وجيزة. لذلك، يمكن القول إنه لا يغير من الصفات الفيزيائية أو الخواص الطبيعية للجبن الأبيض الطري، مما يجعله خياراً مناسباً للاستخدام في هذه المرحلة من الإنتاج دون التأثير سلباً على جودة المنتج

الكلمات الدالة: بكتيريا باسيليس سيرس، الجينات الممرضة، الامراض الناتجه من تناول الغذاء، الماء االمحلل كهربيا المتعادل، الماء المحلل كهربيا القلوى.