

EFFICACY OF NEUTRAL ELECTROLYZED WATER AS DISINFECTANT OF TABLE EGG SHELL ON *SALMONELLA ENTERITIDIS*, *ESCHERICHIA COLI* AND SHELF LIFE OF TABLE EGG.Amal F. El Banna^{1*}, Seham E. Zahran¹, and Eman K. Fathalla¹

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ABSTRACT

Conventional chemical disinfectants used for sanitizing eggs can leave behind toxic residues, which might affect the eggs' ability to hatch. As an alternative to traditional methods of egg sterilization, Neutral Electrolyzed Water (NEW) is gaining popularity. In addition to being efficient and safe, it is also user-friendly, inexpensive, and kind to the environment. This study aimed to determine whether or not different concentrations of Neutral Electrolyzed Water (NEW) might eradicate *Salmonella Enteritidis* and *Escherichia coli* O157 from newly produced eggs. The concentrations tested were 0.1%, 0.3%, 0.6%, 1%, and 3%. The eggs were tested for their internal quality and shelf life at 4°C for 30 days. The tests included albumen pH, yolk pH, yolk index, weight loss, and Haugh unit. Using a 3% concentration of NEW completely killed *S. Enteritidis* and *E. coli* O157 on the eggs' surface. The storage duration had a substantial impact on the quality of all the eggs tested ($P < 0.05$). Yolk index and Haugh unit values fell with increasing storage time, although albumen pH, yolk pH, and weight loss rose. The quality of the albumen and yolk was better retained in the NEW-treated groups compared to the untreated eggs, and weight loss was decreased overall. The results showed that NEW decreased surface corrosion on eggs, which may mean less water and carbon dioxide escaping the eggshell. This contributed to making shelled eggs more microbiologically safe and increasing their storage life.

KEYWORDS: Shelled eggs, Neutral electrolyzed water, Shelf life, Microbial safety.**1. INTRODUCTION**

Eggs are an inexpensive and nutrient-dense meal option. In addition to being one of the healthiest and most versatile meals for humans^[1] and they also contain a wide variety of important nutrients and have many culinary uses.^[2] All of the essential amino acids, phosphates, proteins, and fatty acids found in eggs.^[3] There are three distinct coverings on a newly laid egg: a protective layer of wax, the shell itself, and an inner shell membrane. These layers collectively help to some extent in preventing microorganisms from penetrating the egg^[4] however, these protective layers can still be compromised by various pathogens such as *Salmonella* and *Escherichia coli* during laying (vertical transmission)^[5] or processing, transportation, and storage (horizontal transmission).^{[6][7]} Bacteria such as salmonella can easily infiltrate an egg's inside once it has cracked open^[8] and It is often believed that harmful germs may thrive in egg yolks.^[9] The poultry business stands to lose money if this penetrating factor causes eggs to lose quality while in storage.^[10] The most common bacteria that cause food poisoning are *Salmonella* and *Escherichia coli*.^[11] So, people run the danger of contracting food poisoning if they eat eggs that

are contaminated with *Salmonella* or *E. coli* O157, or if they eat prepared foods that are made with eggs that are infected.^[12]

Several chemical solutions have been developed for the purpose of cleaning and sanitizing eggs in order to aid in the reduction of foodborne infections. Around the world, people use sanitizers that include chlorine to protect their eggshells. On the other hand, there are some serious downsides to using these disinfectants. For example, they remove the eggshell's protective cuticle and release harmful by-products like chloroform, trihalomethanes, chloramines, and haloacetic acids, which are chlorinated compounds. These compounds have the potential to cause cancer and mutations.^{[13][14]} Chlorine is corrosive and is listed under the Directive on Industrial Emissions. Consequently, several European nations have outlawed the use of these substances. These nations include the Netherlands, Germany, Denmark, and Belgium.^{[15][16][17]}

Due to the limits of existing chemical and heat-based methods for microbiological decontamination, as well as the increasing desire for minimally processed foods that are safe, new technologies have been developed to

decontaminate food more effectively and of higher quality. Because of its low cost, ease of use, and lack of negative effects on the environment, electrolyzed water (EW) has become a popular option for microbial decontamination.^[18]

Evidence suggests that electrolyzed water (EW) can eradicate a number of harmful bacteria, such as *Salmonella*, *Escherichia coli* O157, and *Listeria monocytogenes*^[19] and *Bacillus cereus*^{[20] [21]}. It may inhibit the growth of several fungal species and disinfect hepatitis B and human immunodeficiency virus.^[20] EW can also be used in agriculture for sterilization of fruits and vegetables^[22], food items and materials used in food processing.^[19]

Hypochlorous acid (HOCl) is the most prevalent of the chlorine byproducts of the electrolysis process^{[18][23]}, ions of hypochlorite, and chlorine trace amounts.^[24] Neutral electrolyzed water (NEW) bactericidal effect is thought to result from the interaction of these different substances.^[25] These characteristics extend the shelf life of NEW and make it less corrosive than acidic electrolyzed water.^[26] Hypochlorous acid (HOCl) has become significant as an antimicrobial agent across various applications. HOCl is a non-toxic, non-irritating form of chlorine with strong oxidizing abilities. The pH level of the solution is crucial for its production: HOCl reaches its highest effectiveness when the pH is between 5 and 6.5. If the pH exceeds 6.5, the concentration of OCl⁻ increases, and if the pH falls below 5.0, the amount of chlorine gas rises, both of which can reduce the solution's germicidal efficacy.^[27]

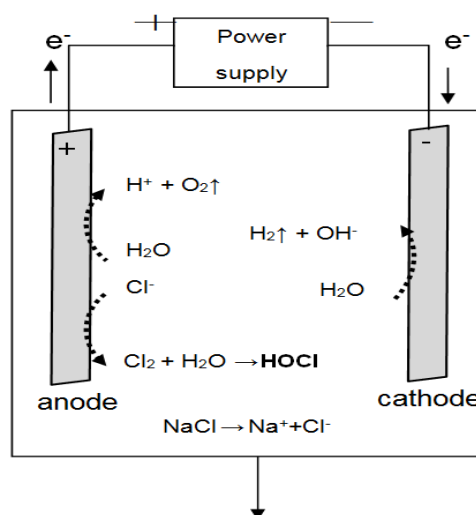
Neutral electrolyzed water (NEW) is widely used to inactivate foodborne bacteria. A number of elements can affect the final NEW product's qualities. These include the machinery utilized, the current settings, the water flow rate, the concentration of acid or salt, the electrolyte and electrode types, the water hardness, and the temperature. Another factor influencing NEW's efficacy is the storage environment in which it is kept.^[28;29]

Besides the eggs themselves, surfaces, and equipment used for grading and packing eggs may be successfully sanitized using NEW in egg processing plants. Instead of using costly and potentially dangerous chemicals, you may clean the surfaces of eggs by dipping them in or spraying them with NEW. This will reduce the risk of food poisoning. The purpose of this study was to determine whether or whether intentionally contaminated shelled eggs could be successfully treated with NEW as an immersion method to eradicate *Salmonella Enteritidis* and *Escherichia coli*. Eggs were also tested for internal quality features including weight loss, albumen pH, yolk index, Haugh unit (HU), and yolk pH when stored at 4°C.

2. MATERIALS AND METHODS

Preparation of Membrane less Electrolyzed Water (MLEW)

The NEW was generated using a manually operated, membrane-free electrolyzing device.^{[30][31]} The device features an 850 ml plastic cylinder filled with NaCl solution at varying concentrations (0.1%, 0.3%, 0.6%, 1%, and 3%). Inside the container, two platinum-titanium electrodes (10 cm x 2 cm) are installed as the anode and cathode, with a 0.8 cm gap among them. The current intensity with thirty minutes of electrolyzing process was 9±2 amp/dm². Obtained NEW were labeled and stored in glass closed containers at refrigerator temperature (4°C). A pH metre fitted with a Garden Grove-based Julle C8 sensory combination pH electrode was used to determine the NEW's pH value).



The schematic diagram of hand-made membrane-less electrolyzing device

Preparation of Bacterial Cultures

E. coli O157 and *S. Enteritidis* strains were acquired from, Animal Health Research Institute (AHRI), Dokki, Egypt. Cultural bacterial population for each tested microorganism was determined according to^[32] performing a tenfold serial dilution of a 0.1 mL aliquot on EMB and XLD media, specific for *E. coli* O157 and *S. Enteritidis*, respectively. For the succeeding trials, the bacterial concentration of the suspensions was increased to 10⁸ colony-forming units (CFU)/mL.

Using the formula:

$$\text{Stock volume} = \frac{(\text{Desired concentration} \times \text{Volume of final suspension})}{\text{Stock concentration}}$$

Design of Experiments

Preparation of Shelled Eggs

A sterile plastic container containing freshly deposited, unfertilized eggs of a certain class weighing 55 to 60 g was brought to the laboratory from the egg farm in Gharbia, Egypt, under regulated temperatures. The tested+ eggs (7 groups for each microorganism including control +ve, control -ve, NEW with concentration 0.1%, 0.3%, 0.6%, 1%, 3% and each group contain 5 eggs)

were initially washed with a 30-milligram-per-liter commercial chlorine sanitizer in tap water for one minute. Following this, they were then cleaned with sterile deionized water to eliminate any remaining sanitizer, after those treated eggs dried under a biosafety hood (ABS1200CLS2-MK2). To prepare the eggs for inoculation, they were submerged in a solution containing 108 CFU/ml of *Escherichia coli* O157 and *Salmonella Enteritidis* for ten minutes. Following that, to promote bacterial adherence, the eggs were left to dry at room temperature (25°C) for 60 minutes.^[33] Shelled eggs were inoculated with *E. coli* O157 and *S. Enteritidis* in separate 500 mL sterile plastic bags. At room temperature (25 ± 2°C), the eggs were left to incubate for three minutes with a solution containing concentrations of 0.1%, 0.3%, 0.6%, 1%, and 3%.

Bacteriological Analysis of Shelled Eggs

The eggs that had been inoculated with *S. Enteritidis* and *E. coli* O157 were put in separate bags of sterile plastic. Each bag contained 500 mL of a new solution with a concentration of either 0.1%, 0.3%, 0.6%, 1%, or 3%. The eggs were left to incubate at a temperature of 25 ± 2°C for a duration of three minutes.^[34]

The experiment was repeated triple using NEW solution with concentrations (0.1%, 0.3%, 0.6%, 1% and 3%) against the most two food borne pathogenic microorganisms (*S. Enteritidis* and *E. coli* O157).

Quality Analysis of Shelled Eggs according to^[33]

Six groups were randomly assigned a total of 108 eggs to be used in the quality test; each group consisted of 18 eggs. There is a control group that does not get any treatment, and then there are other groups that are given varying concentrations of a NEW solution (0.1%, 0.3%, 0.6%, 1%, 3%). The immersion period for each treatment was three minutes. After being correctly marked, the eggs were placed on plastic trays and refrigerated at 4°C for 30 days following treatment. All three egg samples were tested on days 0, 6, 12, 18, 24, and 30 at a temperature of 25 ± 2°C for albumen pH, yolk pH, yolk index, weight loss, and Haugh unit.

Weight Loss

For each egg, an analytical balance with a sensitivity of 0.01 g (XB 220A precisa) was used for weighing. Using the following formula: (i) beginning weight divided by

final weight + (ii) 100Haugh units, we may get the percentage of weight loss during storage. The temperature during storage was kept at 25 ± 2°C.^[35]

PH Measurement

After the albumen and yolk had been separated, they were mixed together in a beaker. The pH levels of albumen and yolks were then determined with a pH meter (Julle C8 Sensory combination Phelectrod Garden Grove, CA92841). We performed the measurements three times and averaged the results. 2°C.

Yolk Index

Using a spatula to crack the eggs onto a smooth glass surface allowed us to examine their internal properties. The diameter of the yolk was measured using a digimatic caliper, and its height was measured using a tripod micrometer. found to be 2.2°C.

Haugh Unit

The Haugh Unit (HU) was calculated using the formula from^[36], using the horizontally distributed albumen and the egg weight as a basis.

For each egg, take its weight (w) and multiply it by the logarithm of the product of its albumen height (h) and its mass (g). This gives you the Haugh unit, which is 100 times the logarithm of the product.

To get the value of h, the tripod micrometer was used to take three separate readings at 10-millimeter intervals around the yolk at various locations inside the thick albumen.

We presented all the parameters as means ± SE after measuring them with three duplicates.

3. RESULTS

Table (1): PH value in the NEW different concentrations the values represent Mean ± SE of three trials.

Group	PH
0.1%	7.60 ± .01
0.3%	7.35 ± .01
0.6%	7.22 ± .02
1%	6.95 ± .01
3%	6.65 ± .02

Table (2): Inactivation of *S. Enteritidis* and *E. coli* O157 on the surface of shell eggs by different concentration of neutral electrolyzed water (NEW) for 3 minutes.

Group	mean ± SE of <i>E. coli</i> O157 count	mean ± SE of <i>S. Enteritidis</i> count
Control +ve	8.20 ± .01 ^a	8.51 ± .01 ^a
0.1%	6.85 ± .01 ^b	7.15 ± .02 ^b
0.3%	5.01 ± .01 ^c	5.72 ± .01 ^c
0.6%	3.46 ± .02 ^d	3.95 ± .03 ^d
1%	1.94 ± .02 ^e	2.44 ± .01 ^e
3%	ND	ND

Mean ± SE of three trials is shown by the values. When different letters follow means within a column, there is a significant difference (p < 0.05).

Table (3): The effect of NEW on the weight loss of the examined egg samples during the storage period at 4°C.

	Control	0.1%	0.3%	0.6%	1%	3%
6days	1.27±0.01 ^a	0.84±0.01 ^b	0.64±0.01 ^c	0.47±0.01 ^d	0.47±0.01 ^d	0.40±0.01 ^e
12 days	1.85±0.03 ^a	1.32±0.06 ^b	1.33±0.03 ^b	1.20±0.05 ^{bc}	1.10±0.05 ^{cd}	1.03±0.03 ^d
18 days	3.03±0.02 ^a	2.40±0.05 ^b	2.1±0.01 ^c	1.8±0.01 ^d	1.72±0.06 ^d	1.73±0.03 ^d
24 days	4.22±0.05 ^a	3.20±0.05 ^b	2.90±0.06 ^c	2.40±0.05 ^d	2.38±0.01 ^d	2.33±0.03 ^d
30 days	5.72±0.06 ^a	4.2±0.04 ^b	4.00±0.05 ^c	3.60±0.06 ^d	3.5±0.05 ^d	3.20±0.05 ^e

The values of weight loss represent Mean ± SE of three trials. Significant differences ($p < 0.05$) exist between means within a column that are followed by different letters.

Table (4): The effect of NEW on the albumin PH of the examined egg samples during the storage period at 4°C.

	Control	0.1%	0.3%	0.6%	1%	3%
Zero	8.2±0.05 ^a	8.16±0.03 ^a	8.2±0.05 ^a	8.2±0.05 ^a	8.2±0.05 ^a	8.2±0.05 ^a
6days	8.38±0.04 ^a	8.35±0.01 ^{ab}	8.31±0.01 ^b	8.3±0.01 ^{bc}	8.29±0.01 ^{bc}	8.24±0.01 ^c
12 days	8.61±0.01 ^a	8.51±0.01 ^b	8.47±0.01 ^c	8.41±0.01 ^d	8.38±0.01 ^e	8.31±0.01 ^f
18 days	8.91±0.01 ^a	8.71±0.01 ^b	8.63±0.01 ^c	8.5±0.01 ^d	8.47±0.01 ^e	8.42±0.01 ^f
24 days	9.1±0.05 ^a	8.94±0.01 ^b	8.81±0.01 ^c	8.75±0.01 ^c	8.66±0.01 ^d	8.6±0.01 ^d
30 days	9.44±0.01 ^a	9.1±0.04 ^b	8.95±0.01 ^c	8.91±0.01 ^{ce}	8.86±0.01 ^{ed}	8.8±0.01 ^e

The values of Albumin PH represent Mean ± SE of three trials. Differences in means within a column that are followed by different letters are statistically significant ($p < 0.05$).

Table (5): The effect of NEW on the yolk PH of the examined egg samples during the storage period at 4°C.

	Control	0.1%	0.3%	0.6%	1%	3%
Zero	6.22±0.01 ^a	6.22±0.01 ^a	6.22±0.01 ^a	6.22±0.01 ^a	6.22±0.01 ^a	6.22±0.01 ^a
6days	6.25±0.01 ^a	6.24±0.01 ^a	6.23±0.01 ^{ab}	6.22±0.01 ^{bc}	6.21±0.01 ^c	6.21±0.01 ^c
12 days	6.34±0.01 ^a	6.28±0.01 ^b	6.26±0.01 ^c	6.25±0.01 ^{cd}	6.24±0.01 ^{de}	6.23±0.01 ^e
18 day	6.36±0.01 ^a	6.32±0.01 ^b	6.3±0.01 ^c	6.29±0.01 ^{cd}	6.28±0.01 ^d	6.26±0.01 ^e
24 day	6.42±0.01 ^a	6.36±0.01 ^b	6.34±0.01 ^c	6.32±0.01 ^c	6.3±0.01 ^d	6.29±0.01 ^d
30 day	6.55±0.01 ^a	6.4±0.04 ^b	6.38±0.01 ^b	6.36±0.01 ^b	6.34±0.01 ^b	6.23±0.01 ^b

The values of yolk PH represent Mean ± SE of three experiments. Differences in means within a column that are followed by different letters are statistically significant ($p < 0.05$).

Table (6): The effect of NEW on the yolk index of the examined egg samples during the storage period at 4°C.

	Control	0.1%	0.3%	0.6%	1%	3%
Zero	0.45±0.01 ^a	0.45±0.01 ^a	0.45±0.01 ^a	0.45±0.01 ^a	0.45±0.01 ^a	0.45±0.01 ^a
6days	0.39±0.01 ^c	0.43±0.01 ^b	0.44±0.01 ^{ab}	0.45±0.01 ^a	0.45±0.01 ^a	0.45±0.01 ^a
12 days	0.3±0.01 ^c	0.42±0.01 ^b	0.43±0.01 ^{ab}	0.44±0.01 ^a	0.44±0.01 ^a	0.45±0.01 ^a
18 days	0.26±0.01 ^e	0.40±0.01 ^d	0.41±0.01 ^{cd}	0.42±0.01 ^{bc}	0.43±0.01 ^{ab}	0.44±0.01 ^a
24 days	0.23±0.01 ^e	0.33±0.01 ^d	0.37±0.01 ^c	0.39±0.01 ^b	0.39±0.01 ^b	0.42±0.01 ^a
30 days	0.22±0.01 ^e	0.30±0.04 ^d	0.34±0.01 ^c	0.36±0.01 ^{bc}	0.37±0.01 ^b	0.40±0.01 ^a

The values of yolk index represent Mean ± SE of three experiments. Differences in means within a column that are followed by different letters are statistically significant ($p < 0.05$).

Table (7): The effect of NEW on the albumin Haugh of the examined egg samples during the storage period at 4°C.

	Control	0.1%	0.3%	0.6%	1%	3%
Zero	82.1±0.6 ^a	82.1±0.6 ^a	82.3±0.6 ^a	82.7±0.2 ^a	82.4±0.3 ^a	82.7±0.1 ^a
6days	75.36±0.5 ^d	76.4±0.5 ^{cd}	78.2±0.5 ^{bc}	79.4±0.5 ^b	81.36±0.3 ^a	81.6±0.8 ^a
12 days	63.66±0.33 ^e	66.73±0.6 ^d	72.4±0.7 ^c	75.5±0.5 ^b	80±0.6 ^a	80.6±0.3 ^a
18 days	59.5±0.5 ^e	61.2±0.5 ^e	69.5±0.7 ^d	72±0.6 ^b	74±0.6 ^b	76±0.5 ^a
24 days	48.5±0.7 ^d	65.5±0.4 ^c	66.4±0.5 ^c	69±0.6 ^b	70.1±0.6 ^{ab}	71.3±0.7 ^a
30 days	40.8±0.4 ^e	55±0.6 ^d	61.2±0.7 ^c	64.36±0.5 ^b	65.3±0.7 ^{ab}	66.5±0.7 ^a

The values of albumin Haugh represent Mean ± SE of three experiments. Differences in means within a column that are followed by different letters are statistically significant ($p < 0.05$).

DISCUSSION

Chemical disinfectants consisting of ozone, sodium hypochlorite, chlorine dioxide, hydrogen peroxide,

organic acids, and chlorinated water have been utilized by the food industry for quite some time, with concentrations varying from 50 to 200 mg/L.^[37]

However, organic compounds have the ability to deactivate chlorine and produce byproducts that might be toxic to humans such as trihalomethanes, halo acetic acids, haloketones, and chloropicrin. These byproducts can be carcinogenic and teratogenic.^[38,39] Food safety and human health depend on finding effective ways to reduce or eliminate germs from eggshells.

In the table (1) pH ranged from 6.65 ± 0.02 at salt concentration of 3% to 7.60 ± 0.01 at salt concentration of 0.1%. The biochemical characteristics of electrolyzed water are affected by the distribution of free available chlorine compounds, which change with pH levels. A single-cell chamber is used to create neutral electrolyzed water (NEW) by combining hydroxide ions (OH⁻) from the negative pole with protons (H⁺) from the positive pole.^[40] and^[41] have confirmed that neutral electrolyzed water typically has a nearly neutral pH range of 6 to 8.

The effects of NEW on shell eggs in terms of killing *S. enteritidis* and *E. coli* O157 are shown in Table (2). The initial viable cell count for *S. enteritidis* was 8.51 ± 0.01 log₁₀ CFU/ml, and for *E. coli* O157 it was 8.20 ± 0.01 log₁₀ CFU/ml. While the viable count recorded after dipping the egg in the different concentration of the NEW 0.1%, 0.3%, 0.6 % and 1 % was 7.15 ± 0.02 , 5.72 ± 0.01 , 3.95 ± 0.03 and 2.44 ± 0.01 log₁₀ CFU/ml respectively for the *S. enteritidis* and was 6.85 ± 0.01 , 5.01 ± 0.01 , 3.46 ± 0.02 and 1.94 ± 0.02 log₁₀ CFU/ml respectively for the count of *E.coli* O157 pointed to the reduction of the viable bacterial count resulted after dipping the egg in the NEW solution for 3 minutes while we could not detect any growth for both *S. enteritidis* and *E. coli* O157 at concentration 3% of NEW for 3 minutes. The reduction in the count increased with increasing the concentration of the NEW. All treatment solutions considerably decreased the population for both *S. enteritidis* and *E. coli* O157, demonstrating that NEW's bactericidal action was effective (P 0.05).

These finding were supported by similar finding of^[42] who demonstrated that NEW had a significantly greater bactericidal effect compared to Citric Acid Solution (CAS) when tested on chicken eggshell surfaces. While CAS only managed a 1.06 log₁₀ CFU/ml decrease in vitro and a 1.74 log₁₀ CFU/egg reduction, NEW managed a 6.11 log₁₀ CFU/ml and 2.18 log₁₀ CFU/egg reduction, respectively.^[43] found that when tested against *Salmonella* Enteritidis and *Escherichia coli* O157, electrolyzed water (EW) with a pH of 5.74 reduced bacterial populations on eggshells by 2.4 and 2.71 log₁₀ CFU/g, respectively.^[44] found that the *E. coli* O157 and *Salmonella* Enteritidis reduction rates were 2.6 log₁₀ CFU/g.

Weight Loss

Weight loss is a crucial measure for assessing quality changes in fresh shelled eggs during storage, as highlighted by.^[45] Table (3) indicates that the relative weight loss of eggs increased with storage time for all

groups kept at 4°C for 30 days. Similar findings were reported by^{[45][46]}, and^[47] who observed significant decreases in egg weight during storage. Possible explanations for the discrepancies in weight loss rates between research include variations in temperature, storage conditions, egg size, hen age, and shell porosity.^[47] Weight loss of eggs during storage is mainly caused by evaporation of water and loss of carbon dioxide from the albumen through the shell's pores.^[45] There were noticeable differences (P < 0.05) among the group of control and the eggs that were treated with NEW and kept at 4°C for 30 days. Weight loss rates for the control group were 5.72 percent, 0.1%, 0.3%, 0.6%, 1%, and 3% NEW-treated eggs were 4.2 percent, 4.4 percent, 3.6 percent, 3.5 percent, and 3.2 percent, respectively. Based on the results of several research, EW with a pH close to neutral has the ability to decrease the egg's weight loss, prevent surface corrosion, and preserve the egg's cuticle.^[48]

PH Measurement

Albumen pH is a good measure of how recently deposited eggs are because it usually ranges from 7.6 to 8.5.^[49] Tables 4 and 5 reveal that the albumen and yolk pH rose dramatically with the passage of time. The albumen pH for all groups was around 8.2 on day 0. The pH of the control group's eggs rose to 9.44 after 30 days at 4°C, whereas the pH of the eggs treated with NEW concentrations of 0.1%, 0.3%, 0.6%, 1%, and 3% reached 9.1, 8.95, 8.91, 8.86, and 8.8, respectively. Compared to eggs treated with NEW, eggs that were not treated had higher albumen pH values (P < 0.05). NEW treatment helped preserve albumen quality by controlling pH levels. This finding aligns with^[35] who noted that CO₂ escapes from the albumen through eggshell pores during storage, leading to pH increases due to changes in the bicarbonate buffer system. Table (5) shows that yolk pH for all groups started at 6.22 and rose to 6.55 in the control group, while it increased to 6.4, 6.38, 6.36, 6.34, and 6.23 for the different NEW concentrations (0.1%, 0.3%, 0.6%, 1%, 3%) after 30 days at 4°C. It is possible that EW's capacity to delay CO₂ release from eggshell pores and decrease cuticle degeneration is responsible for the notable differences between the treated and untreated groups.^[33]

Yolk Index

The yolk index measures the egg's freshness by comparing the yolk's height to its breadth.^[35] Results in table (6) discovered that the yolk index drops dramatically with storage time, hitting a tipping point at 4 degrees Celsius after 30 days. The control group's yolk index value was 0.22 after 30 days of 4°C storage, whereas the groups treated with NEW at concentrations of 0.1%, 0.3%, 0.6%, 1%, and 3% acquired values of 0.30, 0.34, 0.36, 0.37, and 0.40, respectively. Differences between the groups relived that increase the concentration of NEW preserve the egg yolk quality longer than the control eggs. Similar finding recorded by^[46] who brought attention to the changes in the value

of yolk index is freshness indication the vitelline membranes weaken during storage, the total solids decrease, and The main reason the yolk melts is because water from the albumen diffuses osmotically.

Haugh Unit

The height of the inner thick albumen and the egg weight are the two factors that define the HU, and the albumen quality is associated with this height.^[46] The Haugh unit (HU) index of a high-quality, recently-fermented egg is usually about 80, but that of an older egg is usually lower^[49]. As shown in table (7) the HU decreased in all groups during storage after 30 days of storage at 4°C reached to 40.8 in the control group while NEW treated groups had values of 55, 61.2, 64.36, 65.3 and 66.5 respectively for the different applied NEW concentration 0.1 %, 0.3%, 0.6%, 1%, 3%, respectively. In all treatment groups, the NEW decreased the thinning of egg whites while they were stored, leading to higher HU than in the control eggs ($P < 0.05$), irrespective of the concentration. This result is in agreement with^[47] who stated that, at all storage temperatures, the Haugh unit values fell as storage time increased and with^[33] discovered that the disinfectant-treated eggs with SAEW, AEW, and NaClO showed a higher HU after 30 days of storage at 25°C in comparison to the control eggs ($P < 0.05$). The decrease in HU is due to the loss of carbon dioxide gas by the thin albumen height during storage. In turn, this helps raise eggpH by breaking the electrostatic lysozyme-ovomucin complex.^[46]

4. REFERENCES

1. Scott, T. A. and Silversides, F. G. The effect of storage and strain of hen on egg quality. Poult. Sci., 2000; 79: 1725–1729.
2. Dudusola, I. O. Effects of storage methods and length of storage on some quality parameters of Japanese quail eggs. TROPIC- CULTURA, 2009; 27(1): 45–48.
3. Tabidi, M. H. Impact of storage period and quality on composition of table egg. Adv. Environ. Biol., 2011; 5: 856–861.
4. Jay, J.M., Golden, D.A. and Loessner, M.J. Modern Food Microbiology. (7thed.) ISBN: 2005; 978-0-387-23180-8, Publisher: Springer Verlag.
5. Gantois, I, Ducatelle, R, Pasmans, F, Haesebrouck, F, Gast, R, Humphrey, T.J. and Van Immerseel, F. Mechanisms of egg contamination by *Salmonella enteritidis*. FEMS Microbiol. Rev., 2009; 33: 718–738.
6. Davies, R. H. and Breslin, M. Investigation of *Salmonella* contamination and disinfection in farm eggpacking plants. J. Appl. Microbiol., 2003; 94: 191–196.
7. Messens, W., Grijspeerdt, K., De Reu, K., De Ketelaere, B and Mertens, K.: Eggshell penetration of various types of hens eggs by *Salmonella enterica* serovar enteritidis. J. Food Prot., 2007; 70: 623–628.
8. De Reu, K., Messens, W., Heyndrickx, M., Rodenburg, T. B., Uyttendaele, M. and Herman L. Bacterial contamination of table eggs and the influence of housing systems. Worlds Poult. Sci. J., 2008; 64: 5–19.
9. Chousalkar, K. K., Flynn, P., Sutherland, M., Roberts, J. R. and Cheetham, B. F. Recovery of *Salmonella* and *Escherichia coli* from commercial egg shells and effect of translucency on bacterial penetration in eggs. Int. J. Food Microbiol., 2010; 142: 207–213.
10. Holck, A. L., Liland, K. H., Dromtorp, S. M., Carlehog, M. and Mcleod, A. Comparison of UV-C and pulsed UV light treatments for reduction of *Salmonella*, *Listeria monocytogenes*, and *enterohemorrhagic Escherichia coli* on eggs. J. Food Prot., 2018; 81: 6–16.
11. Ebel, E. D., Williams, M. S., Cole, D., Travis, C. C., Klontz, K. C., Golden, N. J. and Hoekstra, R. M. Comparing characteristics of sporadic and outbreak-associated foodborne illnesses, United States, 2004 – 2011. – Emerg. Infect. Dis., 2016; 22(7): 1193.
12. Scott, T. A. and Sweetnam, C. Screening sanitizing agents and methods of application for hatching eggs. I. Environmental and user friendliness. J. Appl. Poult. Res., 1993; 2: 1–6.
13. Bull, R. J., Reckhow, D. A., Li, X., Humpage, A. R., Joll, C. and Hruddy, S. E. Potential carcinogenic hazards of non-regulated disinfection by-products: Haloquinones, halo-cyclopentene and cyclohexene derivatives, N-halamines, halonitriles, and heterocyclic amines. Toxicology, 2011; 286(1–3): 1–19.
14. Legay, C., Rodriguez, M. J., Sérodes, J. B.; and Levallois, P.: Estimation of chlorination by products presence in drinking water in epidemiological studies on adverse reproductive outcomes: A review. Science of the Total Environment, 2010; 408(3): 456–472.
15. Bilek, S. E. and Turantaş, F. Decontamination efficiency of high power ultrasound in the fruit and vegetable industry, a review. International Journal of Food Microbiology, 2013; 166(1): 155–162.
16. Fallik, E. Microbial quality and safety of fresh produce. In W. J. Florkowski, R. L. Shewfelt, B. Brueckner, & S. E. Prussia (Eds.), Postharvest handling. A system approach (3rd rev., pp. 313–339). New York, NY: Elsevier Inc. 2014.
17. Ramos, B., Miller, F. A., Brandão, T. R. S., Teixeira, P. and Silva, C. L. M. Fresh fruits and vegetables – An overview on applied methodologies to improve its quality and safety. Innovative Food Science and Emerging Technologies, 2013; 20: 1–15.
18. Cheng, K. C., Dev, S. R. S., Bialka, K. L. and Demirci, A. Electrolyzed oxidizing water for microbial decontamination of food. Microbial Decontamination in the Food Industry: Novel Methods and Applications, 2012; 563–591.
19. Kim, C., Hung, Y.C., Brackett, R.E. and Lin, C.S. Efficacy of electrolyzed oxidizing water in inactivating *Salmonella* on alfalfa seeds and sprouts. Journal of Food Protection, 2003; 66: 208–214.

20. Buck, J. W., van Iersel, M. W., Oetting, R. D. and Hung, Y. C. In vitro fungicidal activity of acidic electrolyzed oxidizing water. *Plant Disease*, 2002; 86: 278–281.
21. Fabrizio, K.A., Sharma, R.R. and Demirci, A. Comparison of electrolyzed oxidizing water with various antimicrobial interventions to reduce *Salmonella* species on poultry. *Poult. Sci.* 2002; 81(10): 1598–1605.
22. Al-Haq, M. I., Sugiyama, J., and Isobe, S. Applications of electrolyzed water in agriculture & food industries. *Food Science and Technology Research*, 2005; 1: 135–150.
23. Guentzel, J. L., Liang Lam, K., Callan, M. A., Emmons, S. A., and Dunham, V. L. Reduction of bacteria on spinach, lettuce, and surfaces in food service areas using neutral electrolyzed oxidizing water. *Food Microbiology*, 2008; 25(1): 36–41.
24. Liao, L. B., Chen, W. M., and Xiao, X. M. The generation and inactivation mechanism of oxidation-reduction potential of electrolyzed oxidizing water. *Journal of Food Engineering*, 2007; 78(4): 1326–1332.
25. Rahman, S. M. E., Khan, I. and Oh, D. H. Electrolyzed water as a novel sanitizer in the food industry: current trends and future perspectives. *Compr. Rev. Food Sci. Food Saf.*, 2016; 15: 471–490.
26. Rahman, S. M. E., Jin, Y. G. and Oh, D. H.: Combined effects of alkaline electrolyzed water and citric acid with mild heat to control microorganisms on cabbage. *Journal of Food Science*, 2010; 75(2): 111–115.
27. Juhi, S. and Tyler, W. Electrolysed water (hypochlorous acid) generation and efficacy against food-borne pathogens, *Sustainable Food Technol.*, 2023; 1: 603–609.
28. Machado, I., Meireles, A., Fulgêncio, R., Mergulhão, F., Simões, M. and Melo, L. F. Disinfection with neutral electrolyzed oxidizing water to reduce microbial load and to prevent biofilm regrowth in the processing of fresh cut vegetables. *Food Bioprod. Process*, 2016; 333–340.
29. Zhang, C., Li, B., Jadeja, R., Fang, J. and Hung, Y. C. Effects of bacterial concentrations and centrifugations on susceptibility of *Bacillus subtilis* vegetative cells and *Escherichia coli* O157: H7 to various electrolyzed oxidizing water treatments. – *Food Control*, 2016; 60: 440–446.
30. Chuang, C. Y., Yang, S., Huang, H. C., Luo, C. H., Fang, W., Hung, P. C. and Chung, P. R. Applying the membrane-less electrolyzed water spraying for inactivating bio aerosols. *AAQR*, 2013; 13(1): 350–359.
31. Hsu, Y. F., Chuang, C. Y., Huang, H. C. and Yang, S. Applying membrane-less electrolyzed water for inactivating pathogenic microorganisms, applied ecology and environmental research, 2019; 17(6): 15019 -15027.
32. FDA, (2001): Microbiological Methods and Bacteriological Analytical Manual (BAM).
33. Zang, Y. T., Bing, Sh., Li, Y. J., Shu, D. Q., Huang, A. M., Wu, H. X., Lan, L. T. and H. D. Wu. Efficacy of slightly acidic electrolyzed water on the microbial safety and shelf life of shelled eggs, *Poultry Science*, 2019; 98: 5932–5939.
34. Harrigan, W.F. *Laboratory Methods in Food Microbiology*, 3rd ed. Pub. by Academic Press 525 B Street, Suite 1900, San Diego, California, 1998; 92101-4495, USA.
35. Caner, C. and Yuceer, M. Efficacy of various protein-based coating on enhancing the shelf life of fresh eggs during storage. *Poult. Sci.*, 2015; 94: 1665–1677.
36. Haugh, R. R. The Haugh unit for measuring egg quality. *US Poult. Mag.*, 1937; 43: 552–573.
37. Beuchat, L.R., Nail, B.V. and Clavero, M.R.S. Efficacy of spray application of chlorinated water in killing pathogenic bacteria on raw apples, tomatoes and lettuce. *J. Food Prot.*, 1998; 61: 1305–1311.
38. Allende, A., McEvoy, J., Tao, Y. and Luo, Y. Antimicrobial effect of acidified sodium chlorite, sodium chlorite, sodium hypochlorite, and citric acid on *Escherichia coli* O157:H7 and natural microflora of fresh cut cilantro. *Food Control*, 2009; 20: 230–234.
39. Keskinen, L.A. Burke, A. and Annous, B.A. Efficacy of chlorine, acidic electrolyzed water and aqueous chlorine dioxide solutions to decontaminate *Escherichia coli* O157:H7 from lettuce leaves. *Int. J. Food Microbiol.*, 2009; 132: 134–140.
40. Huang, Y. R., Hung, Y. C., Hsu, S. Y., Huang, Y. W. and Hwang, D. F. Application of electrolyzed water in the food industry. *Food Control*, 2008; 19: 329–345.
41. Cui, X., Shang, Y., Shi, Z., Xin, H., and Cao, W. Physicochemical properties and bactericidal efficiency of neutral and acidic electrolyzed water under different storage conditions. *Journal of Food Engineering*, 2009; 91(4): 582–586.
42. Garcia, A. R., Ferro, L. S., Ramirez Orejel, J.C., Agredano Moreno, L. T., Jimenez Garcia, L. F., Paez Esquiliano, D., Esquivel, E. A., Cano Buendia, J. A. The effect of neutral electrolyzed water as a disinfectant of eggshells artificially contaminated with *Listeria monocytogenes*: *Food Sci. Nutr.*, 2019; 7: 2252–2260.
43. Ni, L., Cao, W., Zheng, W., Chen, H. and Li, B. Efficacy of slightly acidic electrolyzed water for reduction of foodborne pathogens and natural microflora on shell eggs. *Food Science and Technology Research*, 2014; 20(1): 93–100.
44. Bialka, K., Demirci, A., Knabel, S., Patterson, P. and Puri, V. Efficacy of electrolyzed oxidizing water for the microbial safety and quality of eggs. *Poultry Science*, 2004; 83(12): 2071–2078.
45. Samli, H. E., Agma, A. and Senkoylu, N. Effects of storage time and temperature on egg quality in old laying hens. *J. Appl. Poult. Res.*, 2005; 14: 548–553.

46. Morsy, M. K., A. M. Sharoba, Khalaf, H. H., Eltanahy, H. H. and C. N. Efficacy of antimicrobial pullulan-based coating to improve internal quality and shelf-life of chicken eggs during storage. *J. Food Sci.*, 2015; 80: 1066–1074.
47. Yimenu, S. M.; Kim, J., Koo, J. and B. Kim Predictive modeling for monitoring egg freshness during variable temperature storage conditions. *Poult. Sci.*, 2017; 96: 2811-2819.
48. Zang, Y. T., B. M. Li, Z. X. Shi, X. W. Sheng, H. X. Wu, and D. Q. Shu. Inactivation efficiency of slightly acidic electrolyzed water against microbes on facility surfaces in a disinfection channel. *Int. J. Agr. Biol. Eng.*, 2017; 10: 23–30.
49. Yuceer, M., Aday M. S. and Caner C. Ozone treatment of shell eggs to preserve functional quality and enhance shelf life during storage. *J. Sci. Food Agric.*, 2016; 96: 2755–2763.