



EVALUATION OF NEUTRAL ELECTROLYZED WATER AS A POTENTIAL FIG PROCESSING SURFACES SANITIZER IN THE FIG INDUSTRY

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Abstract: In this study, the antimicrobial activity of neutral electrolyzed water (NEW) on *Bacillus cereus* forming endospore, *Escherichia coli* and, toxin producer *Aspergillus flavus* and *Penicillium expansum* was determined both on the surface of steel plates in the presence of organic matter artificially inoculated and in cell suspensions. Also, the antimicrobial efficiency of NEW was compared to that of Sodium hypochlorite (NaClO). Experiments were carried out at room temperature (22 °C). 1% sodium hypochlorite solution (with 531 ppm free chlorine), and different concentrations of NEW, 5% (with 63 ppm free chlorine), 10% (with 120 ppm free chlorine), and 15% (with 187 ppm free chlorine) were used for the comparison. Cell suspensions and stainless-steel plates inoculated with a final 10% liquid fig solution were treated with NEW and NaClO for 0 (untreated, control), 15, 30, and 60 seconds. Then, viable cell counts both in cell suspensions and on the inoculated stainless-steel plates were determined. It was determined that there were significant differences ($P<0.05$) in the decrease in the number of microorganisms depending on the application time and free chlorine concentration. The reduction ratios (%) in cell suspensions after 60 seconds of treatment with NEW ranged from 48.8 to 100 for *E. coli*, 11.39 – 32.23 for *B. cereus* and, 31.12 – 100 for *A. flavus*. The reduction ratio for *P. expansum* was %100 for all concentrations of NEW after 60 sec. After 60 seconds application of 1% NaClO to the cell suspensions, the reduction ratios (%) were determined to be 29.56, 23.48, 39.19 and 69.92 for *E. coli*, *B. cereus*, *A. flavus* and *P. expansum*, respectively. However, in the experiments performed after inoculation of microorganisms and sterile 10% liquid fig solution on the surface of steel plates, it was observed that microorganisms showed greater resistance to NEW and 1% NaClO compared to direct application to the cell suspension. The reduction ratios (%) on the surface of steel plates after 60 seconds of treatment with NEW ranged from 17.66 to 40.07 for *E. coli*, 23.93–31.77 for *B. cereus*, 10,91–30,91 for *A. flavus* and, 49.77–64.85 for *P. expansum*. After 60 seconds application of 1% NaClO on the surface of steel plates, the reduction ratios (%) were 19.38, 11.70, 7.5 and 46.52 for *E. coli*, *B. cereus*, *A. flavus* and *P. expansum*, respectively. The results of this study showed that 15% NEW can be used as a strong bactericide and fungicide against endospore-forming bacteria and toxin-producing fungi. Also, 15% NEW is more effective than 1% NaClO in cleaning the surfaces used for fig processing. Therefore, NEW also can be a good alternative to commonly used disinfectants. This is the first report on the use of NEW as a fungicide and bactericide on fig processing surfaces in the fig industry.

Keywords: Neutral electrolyzed water, Fig industry, Endospore-forming bacteria, *E. coli*, *Aspergillus flavus*, *Penicillium expansum*

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1. Introduction

Fig (*Ficus carica* L.), named after Caria, an ancient settlement in the Aegean Region, is a fruit belonging to the Moraceae (mulberry) family. The fig, which homeland is Türkiye, spread to Syria and Palestine, and then to China and India. Türkiye ranks first in the world in fig production and export and exported 20 thousand tons of fresh figs and 57 thousand tons of dried figs in 2019/2020.

During the production of dried figs in Türkiye, the figs are dried on trays in a greenhouse or under sunlight (Aksoy, 1997). Since figs are exposed to outdoor air for a long time on the ground during the drying process, the risk of contamination with pests and pathogens is quite

high (Flaishman et al., 2008). It has been determined that dried figs are contaminated with aflatoxin and ochratoxin A during the preparation, processing, storage, and distribution stages for marketing (Tosun and Delen, 1998). It has been determined that *Aspergillus* species such as *Aspergillus flavus* and *Aspergillus parasiticus* cause aflatoxin formation, and many saprophytic fungi species such as especially *A. niger* and *Penicillium* species cause ochratoxin A formation in dried figs (Heperkan, 2006). Studies on dried figs have shown the presence of *Bacillus cereus* and *Escherichia coli* contaminations in numbers ranging from 10^7 to 10^8 cfu per gram, except for fungi (Akbas and Ozdemir, 2008).

The amounts of aflatoxins detected in export products are very high in the records of the Rapid Alert System for



Food and Feed (RASFF). For example, the amounts of aflatoxins detected in products such as hazelnuts, dried figs and peanuts exported in 2017 are much higher than the amounts allowed by Türkiye's legislation. In Türkiye, 137 lots in 2017 and 110 lots in 2018 of food products were returned due to aflatoxin residues (RASFF, 2017; 2018). These data show that current food safety standards and regulations are insufficient. Therefore, the development of an effective disinfectant in killing pathogenic microorganisms in food production and agriculture is one of the most critical food safety steps for the principles of Hazard Analysis Critical Control Points (HACCP). The presence and proliferation of pathogenic microorganisms in food establishments and health institutions pose great risks to public health (CDC, 2013). The use of chemical agents as disinfectants in food and food processing areas is the most economical and common method to reduce the number of microorganisms that cause foodborne disease risk to acceptable levels (Brasil, 2007). Approved food disinfectants must be safe for use on food contact surfaces, require no rinsing after disinfection, and be free of dyes and fragrances (Gaulin et al., 2011). However, only a few products are allowed for use in food production areas. In addition, there is limited information on the fight against mycotoxin-producing species. Fungicides such as difenoconazole, azoxystrobin, thiabendazole, and copper oxychloride are effective, but over time, fungi develop resistance to these fungicides (Vasquez-Lopez et al., 2021). The most known fungicides, on the other hand, are not environmentally friendly and also leave residues. Hydrogen peroxide (H_2O_2) is an alternative sanitizing agent approved as safe by Food and Drug Administration (FDA), but it has been determined to cause phytotoxicity in both sweet cherry fruit and stem (Sehirli et al., 2020). Since chemical disinfectants used in food and food processing areas have left residues, more sustainable alternatives such as electrolyzed water (EW, electrolytically generated hypochlorous acid or electrochemically activated (ECA) water) have begun to be investigated (Ovissipour et al., 2015). In recent years, EW containing hypochlorous acid (HOCl) has been recommended as a new and promising sanitizer and cleaning agent. In the literature, it has been stated that there are 5 different types of EW according to the pH value. These are neutral EW (pH 7–8), acidic EW (pH 2–3), alkaline EW (pH 10–13), slightly alkaline EW (pH 8–10), and slightly acidic EW (pH 5–6.5) (Rahman et al., 2016). The three germicidal factors of pH, ORP, and available chlorine concentration (ACC), which includes chlorine (Cl_2), HOCl, and OCl, are used to classify the sterilizing methods of EOW. In the EW solutions, $\cdot OH$ and HOCl both show powerful antibacterial properties and antioxidant actions that support oxidative stability. Neutral EW (NEW) is stated to be the most promising among these types. NEW contain several compounds such as hypochlorous acid (HOCl), hypochlorite ions (ClO), chlorine dioxide (ClO_2), and ozone (O_3). NEW

predominantly contains HOCl. HOCl compounds are more active in microbial cell wall penetration and oxidative attacks than ClO^- (Veasey and Muriana, 2016). HOCl infiltrates the membranes of germ cells and produces OH^- , which functions as antimicrobial agents through oxidation.

Chlorine loss of NEW (pH 7; ORP 800–900 mV) is not as fast as that of acid EW due to Cl_2 volatilization (Guentzel et al., 2010) and, NEW causes fewer health problems and has less cytotoxic secondary effects compared to acid EW and sodium hypochlorite (NaOCl) (Wang et al., 2007). The usability of EAW in food production, disinfection of food processing and non-food contact processing surfaces has been investigated by many researchers (Guentzel et al., 2010). NEW is a disinfectant that can be used safely because it has fewer adverse effects on workers' hands, food processing surfaces and human health compared to chlorine gas (Al-Haq et al., 2005). It has been determined that it has the potential to be used successfully after harvest due to its strong effect, low production cost, not producing harmful by-products or leaving residues, and being accepted for use in organic production (Villarreal-Barajas et al., 2022).

Stainless steel is the most commonly used material for food production surfaces in the food industry. Ayebah and Hung (2005) reported that there was no negative effect of EW water on stainless steel surfaces for 8-days.

Although there are many studies focused on the antibacterial properties of NEW, studies focusing on its antifungal activity are not many. Some studies reported that NEW shown promising effects on *Aspergillus* spores isolated from peanut seeds (Xiong et al., 2010), on *Botrytis cinerea* and *Monilia fructicola* (Guentzel et al., 2010), and, on *Fusarium* isolated from cereals (Audenaert et al., 2012). In the same available chlorine concentration level, NEW contains more $\cdot OH$ than acid EW. The $\cdot OH$ is an important fungicidal factor that damages the cellular normal function. The oxidative molecules in NEW destroy microbiological organisms' nucleic acids and enzymes, the cells finally die (Xiong et al., 2010).

This study aims to determine the antimicrobial efficacy of NEW (63, 120, and 187 ppm free chlorine) compared to NaClO (531 ppm free chlorine) on *B. cereus*, *E. coli*, toxin producer *A. flavus* and *P. expansum* in a short time like 15, 30 and 60 seconds both on the surface of steel plates inoculated organic matter (dry fig) and in the cell suspensions.

2. Materials and Methods

2.1. Preparation of Microorganism Cultures

To prepare the suspensions of *B. cereus* ATCC 11778 and *E. coli* ATCC 35218, these bacteria were inoculated on Nutrient Agar (NA, Merck Ltd., Germany) and after incubation at 37°C for 24 h, several colonies of these bacteria were transferred to the tubes containing 10 mL of NaCl solution (0.9%, w v⁻¹) with the sterile inoculation loop and finally the tubes were vortexed using a thermal

mixer. The final cell concentration was adjusted to 10^7 log CFU mL⁻¹. To confirm the number of bacteria in each culture, 0.1 mL portions of appropriately diluted culture were plated on NA. After incubation at 37°C overnight, the number of viable cells was counted and reported as log₁₀ CFU g⁻¹ sample. The prepared bacterial cultures were used in the experiments (Zang et al., 2019).

P. expansum and *A. flavus*, isolated from fig fruits, were cultured on Potato Dextrose Agar plates (PDA, Merck Ltd., Germany) at 25°C for one week. After one week incubation at 25°C, the spores of *P. expansum* and *A. flavus* were collected and suspended in sterile Ringer's solution. After filtering through eight layers of sterile cheese-cloth, the spores were counted and adjusted to a final concentration of 10^7 cells per mL (Spadaro et al., 2002).

2.2. Inoculation

50 g of dried figs were homogenized in sterile distilled water with a blender (Bosch MSM66150) and then, the final volume was completed to 250 mL with sterile distilled water. The prepared 20% liquid dried fig solution was sterilized in an autoclave (Hirayama HG-133) at 121 °C for 20 minutes. 1mL samples from the sterile liquid dried fig solution, which was shaken well, were mixed with 1mL of bacteria and fungal cultures (approximately 10^7 CFU mL⁻¹). Thus, the final ratio of each liquid-dried fig solution became 10% (Zang et al., 2019).

Stainless steel plates (6.0 x 15.0 cm) were purchased from a commercial source. Before inoculation, they were sterilized in an autoclave (Hirayama HG-133) at 121 °C for 20 min after washing thoroughly with tap water. After sterilization, they were dried in a biosafety cabinet for 30 minutes. 0.1 mL of a solution of figs inoculated with microorganisms was spread over a 5 x 5 cm area of the dried stainless steel plates. Then, the inoculated plates were left to dry for 30 minutes at room temperature in a biosafety cabinet so that bacteria and fungi could adhere completely to the surface. Each application was carried out in three parallels and two replications.

2.3. Preparation of NEW

NEW was obtained by electrolysis of a mixture of NaCl (20 g L⁻¹) and tap water using a Stel-10H-120-01 generator (Stel - 10H- 120-01, Russia) at 40.0 V, 9.0 A and a rate of 250 mL 22 sec⁻¹. NEW dilutions were prepared by using sterile tap water, prepared by autoclaving at 121 °C for 15 min, at rates of 5, 10 and 15%. Analytical indices (Oxidation Reduction Potential (ORR), pH and available chlorine concentration (ACC)) of the treated solutions were measured immediately after 5, 10 and 15% NEW preparation. The pH was measured with a pH meter (HI 2211-02, HANNA, USA), and ORP was measured with an ORP meter (HI98120, Hanna, USA). The pH meter was calibrated using commercial standard buffers at pH 4.0 and 7.0 (Merck Ltd., Germany). The ACC was measured based on the iodometric method reported by Dychdala (1983).

2.4. Direct NEW Treatment on Pure Cultures

0.9 mL of 1% NaHOCl or NEW (5, 10 and 15%) was transferred to a sterile tube. 0.1 mL of bacteria and mould cultures were added to the tubes. The cultures were treated with disinfectants for 15, 30 and 60 seconds. Another 0.1 mL liquid was mixed with sterile distilled water as the control. After incubation, 9 mL of neutralizer (0.5% Na₂S₂O₃) was added to each tube and the activity of disinfectants was terminated. After a 5-minute neutralization period, viable bacterial counts were determined by the dilution method. To determine viable cell numbers, NA was used for *B. cereus* ATCC 11778 and *E. coli* ATCC 35218 while PDA was used for *A. flavus* and *P. expansum*. Nutrient agar was incubated at 37 °C for 24 hours while PDA was incubated at 25 °C for 3-5 days (Messer et al., 2000). The sterilization rate was calculated using the below equation.

$$\text{Sterilization rate (\%)} = 100 (\text{Mc-MT})/\text{Mc}$$

Where, Mc is the total number of microbial colonies before disinfection, CFU mL⁻¹; MT is the total number of microbial colonies after disinfection, CFU mL⁻¹.

Each application was carried out in three parallels and two replications. The average values of the outcomes were presented.

2.5. NEW Treatment on Inoculated Steel Plates and Microbiological Analysis

1% NaHOCl (with 531 ppm free chlorine), and different concentrations of NEW, 5% (with 63 ppm free chlorine), 10% (with 120 ppm free chlorine), and 15% (with 187 ppm free chlorine) and sterile physiological water as a control were sprayed separately on the inoculated stainless steel plates. After different application times of 15, 30 and 60 seconds, the samples collected from the surface by wiping 20 times with sterile swabs were transferred to tubes containing 9 mL neutralizer (0.5% Na₂S₂O₃) for microbial analysis. After the tubes were thoroughly mixed with a vortex device at 1500 rpm, the numbers of viable microorganisms were determined by the dilution method. To determine viable cell numbers, NA was used for *B. cereus* ATCC 11778 and *E. coli* ATCC 35218 while PDA was used for *A. flavus* and *P. expansum*. NA was incubated at 37 °C for 24 hours while PDA was incubated at 25 °C for 3-5 days (Messer et al., 2000). The inactivation rate was calculated using the above equation. Each application was carried out in three parallels and two replications. The average values of the outcomes were presented.

2.6. Statistical Analysis

Each application was of complete randomized design and carried out in three parallels and two replications. Results were analyzed by One-way ANOVA using the LSD test (P<0.05) to determine differences in the efficiencies of disinfectants on microbial inactivation. The statistical analyses were performed with the statistical program JMP Pro 11.

3. Results and Discussion

For consumers, food safety is very important. A very important step before bringing food to market is cleaning and sterilization. If food processing surfaces are not cleaned and disinfected effectively, microbial contamination can cause food-borne health problems. Several factors complicate or limit the reduction of microbial load and the application of chemical food disinfectants, such as the nature, species, and initial number of any residual microorganisms on the surface, the nature of the organic and inorganic content present on the surface, the effective dose of the disinfectant, the contact time, presence of chemical residues after disinfection, corrosion on food contact surfaces. Therefore, alternatives to traditional disinfectants have been investigated (Wang et al., 2016).

In recent years, a lot of research has been conducted on electrolyzed water. Most of the studies with EW have focused on gram-positive and negative bacteria that do

not form endospores in food contamination (Al-Qadiri et al., 2016; Ovisipour et al., 2015). But, Al-Qadiri et al., (2019) examined the antimicrobial activity of NEW against endospore-forming *Bacillus cereus* and *Clostridium perfringens* in cell suspensions laboratory inoculated fresh produce and polypropylene cutting board surfaces.

This study might be the first study to show the efficacy of NEW with low ACC concentrations on endospores and toxin-forming microorganisms in such a short time on dried fig-contaminated surfaces

In our study, it was investigated the effectiveness of NEW containing very low concentrations of ACC (63 -187 ppm) (Table 1) in very short periods (15-60 seconds) against toxin-producing moulds (*A. flavus* and *P. expansum*) as well as gram-negative bacteria (*E. coli* ATCC 35218) and gram-positive endospore-forming bacteria (*B. cereus* ATCC 11778) both in pure cultures and on surfaces containing organic matter.

Table 1. Concentration and physicochemical properties of NEW used in the efficacy test

| Concentration (%) | pH | ORP (mV) | ACC (ppm) |
|-------------------|------|----------|-----------|
| 5 | 7.64 | 850 | 63 |
| 10 | 7.62 | 865 | 120 |
| 15 | 7.6 | 880 | 187 |

NEW application showed a broad spectrum effect on the microorganisms, which was used in experiments (Table 2 and 3). It was observed that there were significant differences in the decrease in the number of bacteria and fungi depending on the application time, presence of organic matter and concentration. The maximum inactivation effect was obtained after 60 seconds of application of NEW containing 187 ppm ACC. In the first 15 seconds of application of NEW (5, 10 and 15%) in the cell suspensions, the extents of reductions (%) were 8.95, 25.29, and 28.39 for *E. coli*, 6.78, 7.47, and 32.23 for *B. cereus*, 7.03, 31.12, and 35.25 for *A. flavus*, 63.36, 100 and 100 for *P. expansum*, respectively. The extents of reductions (%) of sodium hypochlorite containing 531 ppm ACC in the same duration were 18.8 for *E. coli*, 9.86 for *B. cereus*, 35.10 for *A. flavus* and 59.14 for *P. expansum*, respectively. 60 seconds application of NEW has a significantly ($P<0.05$) high inhibitory effect when compared with its 15 and 30 seconds application results. The extents of reductions (%) of NEW for 60 seconds were 48.8, 100 and 100 for *E. coli*, 11.39, 13.37 and 32.23 for *B. cereus*, 7.12, 36.52 and 100 for *A. flavus* and 100, 100 and 100 for *P. expansum*, respectively. In the same duration, the extents of reductions (%) of sodium hypochlorite containing 531 ppm ACC were 29.56 for *E. coli*, 23.48 for *B. cereus*, 39.19 for *A. flavus* and 69.92 for *P. expansum*, respectively. Considering the results of the study, it is seen that sodium hypochlorite, which contains 2.84 times more ACC (531 ppm), does not have as much inactivation effect as 15% NEW (187 ppm ACC). In addition, it is seen that there are clear differences in the

resistance of spore-forming bacteria and non-spore-forming bacteria. Kim et al., (2000) showed that within 60 seconds, acid EW (pH 2.5, ORP of 1123 mV, and 10 mg L⁻¹ free available chlorine (FAC)) caused a reduction of 10 log CFU mL⁻¹ in *E. coli* O157:H7 and a reduction of 3 log CFU mL⁻¹ in spore-forming *B. cereus* which was more resistant.

Al-Qadiri et al., (2019) reported that after a minute application of NEW containing 120 mg L⁻¹ FAC on *B. cereus* in the cell suspension, the number of spore-forming *B. cereus* decreased from 5.85 to 3.72 log 10 CFU ml⁻¹, that is, NEW had an inhibiting effect of 36% in the number of spore-forming bacteria. In our study, the inhibition effect of NEW containing 187 ppm ACC on *B. cereus* within 1 minute was 32.23%. It has also been confirmed in previous studies that endospore-forming bacteria are less sensitive to chemicals such as EW and nisin than non-spore-forming bacteria (Kim et al., 2000; Al-Qadiri et al., 2019).

When the rate of microbial inactivation of NEW on the inoculated steel surfaces (Table 3) compares with that of cell suspensions (Table 2), it is seen that the cells in the cell suspensions are more sensitive to NEW. As the reduction ratios (%) on the surface of steel plates after 60 seconds of treatment with NEW ranged from 17.66 to 40.07 for *E. coli*, 23.93–31.77 for *B. cereus*, 10.91–30.91 for *A. flavus* and, 49.77–64.85 for *P. expansum*, the reduction ratios (%) by 1% NaClO were 19.38, 11.70, 7.5 and 46.52 for *E. coli*, *B. cereus*, *A. flavus*, and *P. expansum*, respectively (Table 3). The results of the study showed that the presence of organic matter on the surface has

negative effects on reducing the microbial load and the antimicrobial activity of NEW increased as the ACC concentration increased (Table 2 and 3).

Al-Qadiri et al., (2016) investigated the efficiency of NEW (ORP = 805 mV, pH = 6.6, and ACC = 200 mg L⁻¹) on 5 different non spore-forming bacteria (*Staphylococcus aureus*, *E. coli* O157:H7, *S. typhimurium*, *L. monocytogenes* and *C. jejuni*) inoculated on the surfaces of wooden and polypropylene food cutting boards. The results of the study showed that the contact time, surface properties and the presence of organic matter on the surface have positive or negative effects on reducing the microbial load. Al-Qadiri et al., (2019), in another study, examined the antimicrobial activity of NEW (pH = 6.6, ORP = 805 mV, and ACC= 120 mg L⁻¹) on two different endospore-

forming bacteria (*B. cereus* and *C. perfringens*) inoculated on the surface of a polypropylene cutting board. They found that a 5-minutes application of NEW caused a decrease of 2.33 log CFU / 100 cm² in the number of *B. cereus* and a decrease of 3.06 log CFU / 100 cm² in the number of *C. perfringens*. When we compare the two studies conducted on the surface of food cutting boards by Al-Qadiri et al., in 2016 and 2019, it is seen that NEW is more effective on non-spore-forming bacteria than spore-forming bacteria. In our study, on stainless steel surfaces inoculated with 10% dried fig solution, 15% NEW inactivated *E. coli* at a rate of 40.07% within 60 seconds, while inactivating spore-forming *B. cereus* at a rate of 31.77% (Table 3). These data are similar to the literature.

Table 2. Sterilization effect of NEW and NaClO in cell suspensions*

| Disinfectant | Disinfectant concentration (%) | ACC (ppm) | Treatment solution time (sec.) | Inactivation Rate (%) | | | |
|--------------|--------------------------------|-----------|--------------------------------|--------------------------|----------------------------|----------------------|---------------------|
| | | | | <i>E.coli</i> ATCC 35218 | <i>B.cereus</i> ATCC 11778 | <i>A.flavus</i> | <i>P. expansum</i> |
| NEW | 5 | 63 | 15 | 8.95 ⁱ | 6.78 ^f | 7.03 ^g | 63.36 ^d |
| | | | 30 | 13.92 ^h | 7.29 ^f | 8.39 ^g | 69.36 ^{bc} |
| | | | 60 | 48.80 ^b | 11.39 ^{de} | 7.12 ^g | 100.00 ^a |
| | 10 | 120 | 15 | 25.29 ^e | 7.47 ^f | 31.12 ^f | 100.00 ^a |
| | | | 30 | 100.00 ^a | 9.79 ^e | 33.30 ^{ef} | 100.00 ^a |
| | | | 60 | 100.00 ^a | 13.37 ^{cd} | 36.52 ^{cde} | 100.00 ^a |
| | 15 | 187 | 15 | 28.39 ^d | 32.23 ^a | 35.25 ^{de} | 100.00 ^a |
| | | | 30 | 100.00 ^a | 32.23 ^a | 42.98 ^b | 100.00 ^a |
| | | | 60 | 100.00 ^a | 32.23 ^a | 100.00 ^a | 100.00 ^a |
| NaClO | 1 | 531 | 15 | 18.80 ^g | 9.86 ^e | 35.10 ^{de} | 59.14 ^e |
| | | | 30 | 22.57 ^f | 14.59 ^c | 37.69 ^{cd} | 66.11 ^{cd} |
| | | | 60 | 29.56 ^c | 23.48 ^b | 39.19 ^{bc} | 69.92 ^b |

^{a-i} Indicate the differences in the columns.

*It was grouped in the Ninety-five Percent Confidence Interval with the LSD Test. In random blocks, the project was carried out according to the factorial trial design (2 factors- 1. Factor disinfectants, 2. Factor application time). Since the data are percent values, the square root transformation was applied.

Table 3. Sterilization effect of NEW and NaClO on the surface of steel plates in the presence of organic matter artificially inoculated with a final 10% liquid dried fig solution

| Disinfectant | Disinfectant concentration (%) | ACC (ppm) | Treatment solution time (sec.) | Inactivation Rate (%) | | | |
|--------------|--------------------------------|-----------|--------------------------------|--------------------------|----------------------------|-----------------|---------------------|
| | | | | <i>E.coli</i> ATCC 35218 | <i>B.cereus</i> ATCC 11778 | <i>A.flavus</i> | <i>P. expansum</i> |
| NEW | 5 | 63 | 15 | 2.13 ^e | 18.60 | 10.27 | 40.71 ^g |
| | | | 30 | 7.65 ^d | 18.42 | 10.39 | 41.40 ^g |
| | | | 60 | 17.66 ^c | 23.93 | 10.91 | 49.77 ^{de} |
| | 10 | 120 | 15 | 8.55 ^d | 15.81 | 16.09 | 51.18 ^d |
| | | | 30 | 32.46 ^b | 19.51 | 17.70 | 52.66 ^{cd} |
| | | | 60 | 53.36 ^a | 25.13 | 18.39 | 57.19 ^b |
| | 15 | 187 | 15 | 17.53 ^c | 26.25 | 27.62 | 51.43 ^d |
| | | | 30 | 36.12 ^b | 27.82 | 25.78 | 55.93 ^{bc} |
| | | | 60 | 40.07 ^b | 31.77 | 30.91 | 64.85 ^a |
| NaClO | 1 | 531 | 15 | 15.87 ^c | 9.325 | 8.32 | 46.37 ^{ef} |
| | | | 30 | 18.74 ^c | 10.63 | 7.87 | 45.28 ^f |
| | | | 60 | 19.38 ^c | 11.70 | 7.50 | 46.52 ^{ef} |

^{a-g} Indicate the differences in the columns.

*It was grouped in the Ninety-five Percent Confidence Interval with the LSD Test. In random blocks, the project was carried out according to the factorial trial design (2 factors- 1. Factor disinfectants, 2. Factor application time). Since the data are percent values, the square root transformation was applied.

The presence of mycotoxin-producing fungi in food processing and packaging areas is a matter of concern. They first contaminate food products and then produce toxins. To avoid these downsides, food processing areas, and equipment must be sanitized with an effective antifungal agent using an effective hygiene procedure (Lemos et al., 2020). Sodium hypochlorite is the most commonly used agent in the food industry in Brazil as a sanitizer. It is quite popular in the food industry worldwide. When used alone against mycotoxin-producing fungi in the food industry, sodium hypochlorite is ineffective due to fungal spores (Menegaro et al., 2016). The results of our study showed that 15% NEW (187 ppm ACC) against Sodium hypochlorite containing 531 ppm ACC was more effective on fungal spores (Tables 2 and 3).

Buck et al. (2002) showed that *A. flavus* and *A. niger* spores (5×10^6 conidia mL⁻¹) were completely eliminated within 30 seconds by acid EW (54-56 ppm ACC). Xiong et al., (2010) demonstrated that NEW was more effective than acid EW in the inhibition of *A. flavus* spores. It was determined by Yamaner et al., (2016) that 5% NEW at 50°C caused a reduction of 5.54 log CFU ml⁻¹ in the number of *A. flavus* spores and a reduction of 7 log CFU ml⁻¹ in the number of *P. expansum* spores in one minute. It is seen that *P. expansum* spores are more sensitive to NEW compared to *A. flavus* spores. These results show parallelism with the data of our study (Tables 2 and 3).

Lemos et al., (2020) determined that the effectiveness of acid (pH 2.67) and basic (pH 11.29) electrolyzed water (ACC 121 ppm) on toxigenic *Aspergillus* species was low (reduction rate of *Aspergillus* species < 3 log CFU g⁻¹). In our study, although a 100% inactivation rate was reached in 60 seconds when 15% NEW was applied directly to *A. flavus* and *P. expansum* spores, the inactivation rates were 30.91% for *A. flavus* and 64.85% for *P. expansum* in the presence of organic material (10% dried fig liquid) on the stainless steel surface under the same conditions. When 1% NaClO was applied to stainless steel surfaces for 60 seconds, it caused an inactivation of 7.5% in the number of *A. flavus* and 46.52% in the number of *P. expansum*. Therefore, NEW is a very good potential disinfectant compared to NaClO in the presence of organic matter, especially in the food industry.

Preventing fungal contaminations and minimizing cross-contaminations before and after harvest is vital (Al-Haq et al., 2005). The use of NEW, a new and promising disinfectant and cleaning agent, in surface cleaning minimizes corrosion and reduces the negative risks associated with the use of conventional decontaminating agents (Guentzel, 2008). It has also become popular in the food industry in recent years (Villarreal-Barajas et al., 2022).

Electrolyzed water is widely used as an antibacterial agent in many fields such as agriculture, animal husbandry, food sanitation, medicine, etc. EW has been approved for use as a food sanitizer in Russia, Japan and

China. The fact that EW is 100% organic, non-toxic, and low-cost makes its use widespread in both households and industrial applications. The results of this study showed that NEW can be used effectively in the disinfection of fig processing surfaces in the fig industry.

4. Conclusion

Fig infection by toxigenic fungi reported in several studies. Sanitation of food processing surfaces is very important in preventing cross-contamination. But, no study has been conducted related to the use of electrolyzed water treatments as a fungicide and bactericide on fig processing surfaces in the fig industry. This is the first study in this field. The results of this study showed that 15% NEW can be used effectively in the disinfection of fig processing surfaces in the fig industry.

Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

| | Ç. Y. | R.K. |
|-----|-------|------|
| C | 100 | |
| D | 100 | |
| S | 80 | 20 |
| DCP | 90 | 10 |
| DAI | 50 | 50 |
| L | 80 | 20 |
| W | 100 | |
| CR | 90 | 10 |
| SR | 100 | |
| PM | 80 | 20 |
| FA | 80 | 20 |

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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References

- Akbas MY, Ozdemir M. 2008. Application of gaseous ozone to control populations of *Escherichia coli*, *Bacillus cereus* and *Bacillus cereus* spores in dried figs. *Food Microbiol*, 25: 386-391. <https://doi.org/10.1016/j.fm.2007.09.007>.
- Aksoy U. 1997. Harvest and drying of figs. In Aksoy U, editor. *Advanced course on fig production*. Ege University, Izmir, Türkiye, pp: 16-40.
- Al-Haq MI, Sugiyama J, Isobe S. 2005. Applications of electrolyzed water in agriculture & food industries. *Food Sci Technol Res*, 11(2): 135-150. <https://doi.org/10.3136/fstr.11.135>.
- Al-Qadiri MM, Ovissipour N, Al-Alami N, Govindan BN, Shiroodi SG, Rasco B. 2016. Efficacy of neutral electrolyzed water, quaternary ammonium and lactic acid-based solutions in controlling microbial contamination of food cutting boards using a manual spraying technique. *J Food Sci*, 81(5): 1177-1183. <https://doi.org/10.1111/1750-3841.13275>.
- Al-Qadiri HM, Smithc S, Sielaff A, Govindan BN, Ziyainae M, Al-Alamif N, Rascoe B. 2019. Bactericidal activity of neutral electrolyzed water against *Bacillus cereus* and *Clostridium perfringens* in cell suspensions and artificially inoculated onto the surface of selected fresh produce and polypropylene cutting boards. *Food Control*, 96: 212-218. <https://doi.org/10.1016/j.FOODCONT.2018.09.019>.
- Audenaert K, Monbaliu S, Deschuyffeleer N, Maene P, Vekeman F, Haesaert G, De Saeger S, Eeckhout M. 2012. Neutralized electrolyzed water efficiently reduces *Fusarium* spp. in vitro and on wheat kernels but can trigger deoxynivalenol (DON) biosynthesis. *Food Control*, 23 (2): 515-521. <https://doi.org/10.1016/j.foodcont.2011.08.024>.
- Ayebah B, Hung Y. 2005. Electrolyzed water and its corrosiveness on various surface materials commonly found in food processing facilities. *Food Sci Technol*, 28: 247-264. <https://doi.org/10.1111/j.1745-4530.2005.00424.x>.
- Brasil, 2007. Resolução de diretoria colegiada – RDC N° 14, de 28 de fevereiro de 2007 Agência Nacional de Vigilância Sanitária (ANVISA).
- Buck JW, Van Iersel MW, Oetting RD, Hung YC. 2002. *In vitro* fungicidal activity of acidic electrolyzed oxidizing water. *Plant Dis*, 86 (3): 279-281. <https://doi.org/10.1094/PDIS.2002.86.3.278>.
- CDC Centers for Disease Control and Prevention (2013): Surveillance for foodborne disease outbreaks-United States, 2009-2010. *MMWR*, 62: 41-47.
- Dychdala GR. 1983. Chlorine and chlorine compounds. In Block SS, editor. *Disinfection, Sterilization, and Preservation*. Lea and Febiger, Philadelphia, USA, 3rd ed, pp: 157-182.
- Flaishman MA, Rodov V, Stover E. 2008. The fig: Botany, horticulture and breeding. *Hortic Rev*, 34:113-196. <https://doi.org/10.1002/9780470380147.ch2>.
- Gaulin C, Le ML, Shum M, Fong D. 2011. Disinfectants and sanitizers for use on food contact surfaces. National Collaborating Center for Environmental Health, Available http://www.nceh.ca/sites/default/files/Food_Contact_Surface (access date: December 15, 2022).
- Guentzel JL, Lam KL, Callan MA, Emmons SA, Dunham VL. 2008. Reduction of bacteria on spinach, lettuce, and surfaces in food service areas using neutral electrolyzed oxidizing water. *Food Microbiol*, 25(1): 36-41. <https://doi.org/10.1016/j.fm.2007.08.003>.
- Guentzel JL, Lam KL, Callan MA, Emmons SA, Dunham VL. 2010. Postharvest management of gray mold and brown rot on surfaces of peaches and grapes using electrolyzed oxidizing water. *Int. J Food Microbiol*, 143 (1-2): 54-60. <https://doi.org/10.1016/j.ijfoodmicro.2010.07.028>.
- Heperkan D. 2006. The importance of mycotoxins and a brief history of mycotoxin studies in Turkey. *ARI*, 54: 18-27.
- Kim C, Hung YC, Brackett RE. 2000. Efficacy of electrolyzed oxidizing (EO) and chemically modified water on different types of foodborne pathogens. *Int J Food Microbiol*, 61 (2-3): 199-207. [https://doi.org/10.1016/s0168-1605\(00\)00405-0](https://doi.org/10.1016/s0168-1605(00)00405-0).
- Lemos JG, Stefanello A, Olivier Bernardi A, Valle Garcia M, Nicoloso Magrini L, Cichoski AJ, Wagner R, Venturini Copetti M. 2020. Antifungal efficacy of sanitizers and electrolyzed waters against toxigenic *Aspergillus*. *Food Res Int*, 137: 109451. <https://doi.org/10.1016/j.foodres.2020.109451>.
- Menegaro A, Flores AF, Simer P, Silva FI, Sbardelotto PRR, Pinto EP. 2016. Sanitizantes: concentrações e aplicabilidade na indústria de alimentos. *Sci Agrary Parana*, 15: 171 -174. <https://doi.org/10.18188/sap.v15i2.13022>.
- Messer JW, Rice EW, Johnson CH, William MG. 2000. Spread plate technique. In Robinson RK, Batt CA, Patel PD, editors. *Encyclopedia of food microbiology*. Academic Press, New York, USA, pp: 2156-2160.
- Ovissipour M, Al-Qadiri HM, Sablani SS, Govindan NB, Al-Alami N, Rasco B. 2015. Efficacy of acidic and alkaline electrolyzed water for inactivating *Escherichia coli* O104:H4, *Listeria monocytogenes*, *Campylobacter jejuni*, *Aeromonas hydrophila*, and *Vibrio parahaemolyticus* in cell suspensions. *Food Control*, 53: 117-123. <http://dx.doi.org/10.1016/j.foodcont.2015.01.006>.
- Rahman SME, Khan I, Oh DH. 2016. Electrolyzed water as a novel sanitizer in the food industry: Current trends and future perspectives. *Compr Rev Food Sci*, 15: 471-490. <https://doi.org/10.1111/1541-4337.12200>.
- RASFF. 2017. Rapid alert system for food and feed. Annual Report 2017 on Functioning of the RASFF, EU Report, EU Commission, Health and Consumer Protection Directorate-General. <https://op.europa.eu/en/publication-detail/-/publication/f4adf22f-4f7c-11e9-a8ed-01aa75ed71a1/language-en> (access date: December 15, 2022).
- RASFF. 2018. Rapid alert system for food and feed. Annual Report 2018 on Functioning of the RASFF, EU Report, EU Commission, Health and Consumer Protection Directorate-General. <https://op.europa.eu/en/publication-detail/-/publication/c3318331-d9c4-11e9-9c4e-01aa75ed71a1/language-en> (access date: December 15, 2022).
- Sehirli S, Karabulut OA, Ilhan K, Sehirli A. 2020. Use and efficiency of disinfectants within a hydrocooler system for postharvest disease control in sweet cherry. *Int J Fruit Sci*, 20 (S3): 1590-1606. <https://doi.org/10.1080/15538362.2020.1822265>.
- Spadaro D, Vola R, Piano S, Gullino ML. 2002. Mechanism of action and efficacy of four isolates of the yeast *Metschnikowia pulcherrima* active against postharvest pathogens on apples. *Postharvest Biol Technol*, 24: 123-134. [https://doi.org/10.1016/S0925-5214\(01\)00172-7](https://doi.org/10.1016/S0925-5214(01)00172-7).
- Tosun N, Delen N. 1998. Minimising of contamination of aflatoxigenic fungi and subsequent aflatoxin development in fig orchards by fungicides. *Acta Hort*, 480: 193-197.
- Vasquez-Lopez A, Gomez-Jaimes R, Villarreal-Barajas T. 2021. Effectiveness of neutral electrolyzed water and copper oxychloride on fungi spores isolated from tropical fruits.

- Heliyon, 7: e07935. <https://doi.org/10.1016/j.heliyon.2021.e07935>.
- Veasey S, Muriana PM. 2016. Evaluation of electrolytically-generated hypochlorous acid ('Electrolyzed Water') for sanitation of meat and meat-contact surfaces. *Foods*, 5(2): 42. <https://doi.org/10.3390/foods5020042>.
- Villarreal-Barajas T, Vázquez-Durán A, Méndez-Albores A. 2022. Effectiveness of electrolyzed oxidizing water on fungi and mycotoxins in food. *Food Control*, 131: 108454. <https://doi.org/10.1016/j.foodcont.2021.108454>.
- Wang L, Bassiri M, Najafi R, Najafi K, Yang J, Khosrovi B, Robson MC. 2007. Hypochlorous acid as a potential wound care agent: Part I. Stabilized hypochlorous acid: A component of the inorganic armamentarium of innate immunity. *J Burns Wounds*, 6(e5): 65-79.
- Wang X, Demirci A, Puri VM. 2016. Electrolyzed Oxidizing Water for Food and Equipment Decontamination. In: *Handbook of Hygiene Control in the Food Industry*. Elsevier Inc., Woodhead Publishing, New York, USA, 2nd ed., pp: 503-520.
- Xiong K, Liu HJ, Liu R, Li LT. 2010. Differences in fungicidal efficiency against *Aspergillus flavus* for neutralized and acidic electrolyzed oxidizing waters. *Int J Food Microbiol*, 137(1): 67-75. <https://doi.org/10.1016/j.ijfoodmicro.2009.10.032>.
- Yamaner Ç, Ayvaz M, Konak R, Tan N, Kösoğlu İ, Dimoglo A. 2016. Efficacy of neutralised electrolysed water and mild heat against foodborne pathogens isolated from *Ficus carica*. *Ital J Food Sci*, 28(2): 208-220. <https://doi.org/10.14674/1120-1770/ijfs.v260>.
- Zang YT, Bing S, Li YJ, Shu DQ. 2019. Application of slightly acidic electrolyzed water and ultraviolet light for *Salmonella enteritidis* decontamination of cell suspensions and surfaces of artificially inoculated plastic poultry transport coops and other facility surfaces. *Poultry Sci*, 98: 6445-6451. <http://dx.doi.org/10.3382/ps/pez520>.