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Synergistic effect of oxidized electrolyzed water and fumaric acid for control of some species of *Salmonella enterica* in beef.

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ABSTRACT

Salmonella is the common reason of foodborne concern in meat and meat products worldwide. Chemical sanitizers as fumaric acid (FA) and the slightly acidic electrolyzed water (SAEW) can reduce microbial contaminations which reflected on the quality and microbial safety of beef. The current study was performed to determine the synergetic impact of 1% FA and SAEW 30 mg/L for the deactivation of both *Salmonella enteritidis* and *Salmonella virchow* previously isolated from meat. A two-kilogram beef sample was divided into two portions, each inoculated with a different *Salmonella* strain: *S. virchow* and *S. enteritidis*, both of 10^6 CFU/ml. Each portion was subdivided into four groups; group A (positive control), group B (treated with SAEW 30 mg/L pH 6 for five minutes), group C (treated with FA 1% for five minutes), and group D (treated with SAEW 30 mg/L for five minutes then FA 1% for five minutes). Results indicated that SAEW 30mg/L caused a log reduction of 1.94 log cfu/g (30.9%) and 2.15 log cfu/g (33.3%) in *S. virchow* and *S. enteritidis* respectively. While 1% FA yielded a log reduction of 1.69 log cfu/g (26.9%) in *S. virchow* and 1.83 log cfu/g (28.5%) in *S. enteritidis*. On the other hand, the incorporation of SAEW 30 mg/L and 1% FA led to a log reduction of 2.93 log cfu/g (46.7%) and 3.14 log cfu/g (48.7%) in *S. virchow* and *S. enteritidis* respectively. No noticeable changes in the odor, color, texture, pH and overall appearance (OAA) of beef specimens inoculated with *S. virchow* or *S. enteritidis* after treatment with sanitizing agents in comparison to the control groups. SAEW 30 mg/L and 1% FA have strong bactericidal activity against *Salmonella* spp. The integrated treatment by SAEW and 1% FA exhibited a greater bactericidal impact and significantly reduced the *Salmonella* count in meat compared to single treatments. Despite the strong effect of the electrolyzed water and fumaric acid compound, these compounds were unable to completely eliminate *Salmonella* bacteria, which is considered one of the basic requirements for any food standard specification.

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INTRODUCTION

Because of its rich nutrient content, meat plays a crucial role in a healthy, balanced diet. It is a principal origin of proteins, iron, vitamins, and trace elements (**Pereira and Vicente 2013**).

Despite its benefits, meat may harbor some foodborne pathogens that can be hazardous to humans depending on the concentration of microorganisms and consumer resistance. Foodborne pathogenic organism contaminating meat and its products has been a challenging public health issue for many years (**Ehuwa, Jaiswal, and Jaiswal 2021**).

The steps of processing, transportation, and preservation could be implemented in contamination of meat and meat products. Meat has a high water content, making it a good media for contamination by a wide range of microorganisms. *Salmonella* enterica endures to be the most common bacterial pathogen causing foodborne illness (**Control and Prevention 2009**). *Salmonella* was encountered as the prime causative pathogen in the majority of notified foodborne outbreaks affecting people in the European Union countries (**Popa and Papa 2021**). Various chemical disinfectants were investigated to control microbial contamination, extend shelf life, and ameliorate the microbiological safety and quality of meat during processing and storage (**Lee and Yoon 2021**). Electrolyzed water (EW), chlorinated solutions, salts, and organic acids, alone or in integration were able to minimize microbial contaminations of meat (**Chen et al. 2012**).

Electrolyzed water (EW) was documented to have a potent bactericidal action against most pathogenic microorganisms and was approved as a safe, rapid green technology that can be produced on-site. It is low cost antimicrobial treatment so would be economic (**Jadeja and Hung 2014**). Electrolyzed water is induced by running an electric current via a diluted solution of salt water by usage of membrane cell to yield a strong acidic electrolyzed water, while non-membrane cells output slightly acidic electrolyzed water (SAEW),

with dilute hypochloric acid. SAEW, with an available chlorine concentration between 10 and 30 mg/L and a pH level of 5.0 to 6.5, is approved by the Japanese Ministry of Health and Welfare as a safe food sanitizer (**Suzuki et al. 2002**). Because SAEW has a low content of chlorine, its use reduces the processing equipment's corrosion and skin irritation. Consequently, it's recognized as an eco-friendly sanitizer in parallel to other conventional chemical alternatives (**Kim, Hung, and Brackett 2000**).

Electrolyzed water comes in two main types, defined by their pH: strongly acidic (pH 2.2–3.5) and slightly acidic (pH 5.0–6.5). Slightly acidic electrolyzed water (SAEW), with its near-neutral pH, primarily contains hypochlorous acid (HOCl), which is a far more potent sanitizer than hypochlorite ions at the same concentration about 80 times (**Kim, Hung, and Brackett 2000**).

SAEW sanitization efficacy has been documented to successfully deactivate foodborne pathogens such as *Salmonella* spp. on variable agricultural products, involving vegetables and fruits (**Forghani and Oh 2013**) in addition to beef, egg, and poultry meat (**Jadeja and Hung 2014**). It has also been documented as an effective disinfectant of the surfaces of different food processing utensils (**Deza, Araujo, and Garrido 2005**).

Organic acids are broadly utilized in food manufacturing as chemical preservatives. They proved to have antibacterial action across wide scope of food types and are globally accepted as safe for human consumption (**Mani-López, García, and López-Malo 2012**). The advantage of organic acids' addition as antimicrobial substances in food products relies on some vital parameters such as their chemical formula, molecular weight, physical form, and minimum inhibitory concentration. Other factors include the virulence of the pathogen, acid-food contact time and buffering criteria of the food. In the meat industry, meat dipping into a solution containing organic acid improves fresh meat color and stability and maintains the brightness and extend the meat shelf-life (**Coban 2020**).

Fumaric acid (FA) is considered one of popular organic acids used as antimicrobial additives in meat. It possesses a potent killing action against foodborne bacteria. Fumaric acid is mostly defined as safe by the FDA and is asserted for use in many countries (He et al. 2020). Fumaric acid demonstrates antimicrobial efficacy in meat preservation, effectively minimizing bacterial populations and extending shelf-life. Therefore, while FA can be a valuable component in meat preservation strategies, its concentration and application methods should be carefully optimized to balance microbial safety with the maintenance of desirable meat quality attributes (Zhao et al. 2022). Therefore, the current study was carried out to assess the synergetic effect of SAEW 30 mg/L and 1% FA for the inactivation of *Salmonella* enteritidis and *Salmonella* virchow previously isolated from meat.

MATERIAL AND METHODS

This work was performed from October 2024 to January 2025 in the Bacteriology Department, Animal Health Research Institute, Sohag branch.

Preparation of bacterial cultures

S. enterica subspecies *enterica* serovar *virchow*, and *S. enterica* subspecies *enterica* serovar *enteritidis*, previously recovered from meat samples were obtained from the Department of Bacteriology, Animal Health Research Institute. Each strain was subcultured onto tryptic soy agar (Oxoid) plates and incubated for twenty four hours at 37 °C. Colonies (3-4)

were picked up and inoculated in tryptic soy broth (Oxoid) and then incubated for 2-6 hours at 37 °C. Suspension turbidity was balanced to coordinate with 0.5 McFarland standards and then diluted to get a concentration of 10^6 CFU / ml approximately (Tango et al. 2014).

Preparation of sanitizing solutions

SAEW 30 mg/L employed in our study was brought from the Faculty of Science, Sohag University, Egypt. It was prepared by electrolysis of 6% Hydrochloric acid solutions via an electrolysis apparatus with pH 5.0–6.5. The crystalline FA (Anmol Chemicals Co., USA) was dissolved in one liter of deionized water to obtain a conc. of 1% FA solution (w/v), stored at 50°C from preparation and during the experiment time to avoid its precipitation. The oxidation-reduction potential (ORP) and pH of SAEW and FA were assessed with adual-scale pH meter (PHS-3DW, Labitex, China) holding pH and ORP electrodes. A colorimetric method was used to determine the available chlorine conc. (ACC) via a digital chlorine assay kit (AE86060, Labitex, China). The detection border was 1-300 mg/L. The physic-chemical characteristics of the examined antimicrobial sol. are listed in Table A.

Table A. The physicochemical characteristics of the antimicrobial substances.

| | pH ** | ORP ** | ACC ** |
|-----------|--------------|---------------|---------------|
| SAEW | 6.14 ± 0.067 | 821.1 ± 7.492 | 30.03 ± 0.149 |
| 1%FA | 2.60 ± 0.064 | 590.3 ± 7.382 | ND |
| SAEW+1%FA | 2.93 ± 0.054 | 1086 ± 5.707 | 15.8 ± 0.369 |

**High statistical significant differences between the different treatments according to pH (Kruskal-Wallis statistic= 18.59, p=0.001), ORP (F= 8553, p=0.0001), ACC (t=101.2, p=0.001), and ND means not detected.

Collection and preparation of samples

Two kilograms of boneless beef samples was purchased from butcher shops in Sohag City in clean sterile bags and transported immediately to the laboratory in ice boxes to be prepared and examined as soon as possible. Sample was divided into pieces using a sterile knife. Aliquots weighing 10 ± 0.3 gm were used for the *Salmonella* count, and those weighing 25 ± 0.3 gm were used for measuring the pH value and sensory analysis.

Prior to the experiments, the meat surface was subjected to ultraviolet light (UV) and emitted at 254 nm for 15 min to reduce background microflora (Mansur et al. 2015). Beef samples were divided into two portions, the first portion was inoculated with *S. virchow*, and the second one was inoculated with *S. enteritidis* with a conc. of 10^6 CFU/ml each. The inoculated beef samples were left for one hour at room temperature to help adherence of bacteria, and then subjected to the employed treatments. Each portion was subdivided into four groups; group A (positive control), group B (treated with SAEW 30 mg/L pH 6 for five minutes), group C (FA 1% for five minutes), and group D (SAEW 30 mg/L for five minutes then FA 1% for five minutes).

Thereafter, each 10 g beef sample was mixed with ninety milliliter of buffered peptone water (BPW) (Oxoid) and homogenized for two minutes in a Sward stomacher (400 Circulator, Seward, London, US). After that, to enumerate *Salmonella* spp., ten-fold serial dilutions from the four meat groups were prepared using BPW, and 0.1 mL of diluents were spread onto XLD agar (Oxoid) plates. The inoculated plates were incubated at 37 °C for twenty four hours and then *Salmonella* count was recorded. Each autonomous trial was in triplicates (Hantash et al. 2020).

pH measurement:

pH was measured according to (Feldsine, Abeyta, and Andrews 2002). To determine pH, ten grams beef sample was blended with one hundred milliliters DW and then filtered. The pH of the resulted filtrate was measured using a Crison pH meter (Model 507, Crison,

Barcelona, Spain) with combination electrode (Cat. n°. 52, Crison, Barcelona, Spain) previously calibrated with buffers, pH 7 and 4.

Sensory assessment

According to (Turner et al. 2019), the organoleptic examination of beef was performed for odor, color, texture, pH and overall and overall acceptability with the help of the Animal Health Research Institute, Sohag branch committee composed of 11 staff members. The scale points range was graded as poor (1) acceptable (2), good (3), very good (4), and excellent (5).

Statistical analysis

Data represented as mean, median, and standard deviation of the log mean (SD), the analysis of variables were achieved using MedCalc® Statistical Software version 20 (MedCalc Software Ltd, Ostend, Belgium; 2021). To value whether the data met the suppositions of the statistical approach, the Shapiro-Wilk normality test was used, while Levene's test was used to assess the Homogeneity of Variance Test.

Ordinary ANOVA test with Conover Multiple Comparison Test for Post hoc (no normality in data distribution) were used to elucidate the significant variations between the groups. Obtained data were deemed statistically significant if the p-value was below 0.05, and highly significant if it was below 0.01 (Lantz, Andersson, and Manfredsson 2016).

RESULTS

The initial count of *S. virchow* and *S. enteritidis* in beef samples used to evaluate the sanitizing efficiency of examined treatments sole or in together were approximately 6.27 and 6.42 log cfu/g. Antimicrobials significantly reduced the pathogen ($P < 0.0001$) to 4.33 and 4.28 log cfu/g of *S. virchow* and *S. enteritidis* when treated with SAEW 30 mg/L, and to 4.58 and 4.59 log cfu/g, when dealt with 1% FA, and to 3.34 and 3.29 log cfu/g when treated with the integration of SAEW 30 mg/L and 1% FA (Table 1).

Table 1. Effect of antimicrobials on *S. virchow* and *S. enteritidis* mean counts (log cfu/g± SD).

| Experiment Group | <i>S. virchow</i> | | <i>S. enteritidis</i> | |
|------------------|-------------------|--------|-----------------------|--------|
| | Mean | SD | Mean | SD |
| Control | 6.27 ^a | ±0.020 | 6.42 ^a | ±0.010 |
| SAEW | 4.33 ^b | ±0.061 | 4.28 ^b | ±0.045 |
| 1% FA | 4.58 ^b | ±0.040 | 4.59 ^b | ±0.015 |
| SAEW + 1% FA | 3.34 ^c | ±0.086 | 3.29 ^c | ±0.031 |

There are a significance difference ($P < 0.05$) between means having a different super-scripted small letters.

SAEW 30mg/L caused a log reduction of 1.94 log cfu/g (30.9%) and 2.15 log cfu/g (33.3%) in *S. virchow* and *S. enteritidis*, while

1% FA yielded a log reduction of 1.69 log cfu/g (26.9%) and 1.83 log cfu/g (28.5%) in *S. virchow* and *S. enteritidis* respectively. The incorporation of SAEW 30 mg/L and 1% FA lead to a log reduction of 2.93 log cfu/g (46.7%) and 3.14 log cfu/g (48.7%) in *S. virchow* and *S. enteritidis* respectively (Table 2).

Table 2. Log reduction and percentage of *S. virchow* and *S. enteritidis* treated with different agents.

| Antimicrobial agents | <i>S. virchow</i> | | <i>S. enteritidis</i> | |
|----------------------|-------------------|-----------------|-----------------------|-----------------|
| | Rate | Log Reduction % | Rate | Log Reduction % |
| SAEW | 1.94 | 30.9% | 2.15 | 33.3% |
| 1%FA | 1.69 | 26.9% | 1.83 | 28.5% |
| SAEW + 1% FA | 2.93 | 46.7% | 3.14 | 48.7% |

No noticeable changes in the odor, pH and OAA of beef specimens inoculated with *S. virchow* or *S. enteritidis* after treatment with sanitizers in comparison to the control. Groups treated with sanitizing agents presented a more clearly intense pinker color than the control group. Regarding the texture, treatments produced a slight rise in firmness and work of cutting (table 3).

Regarding *S. virchow*, there were no statistically significant variations among the different treatments according to the pH of the samples ($F= 0.3889$, $p=0.76$). No statistically significant variations among the different treat-

ments according to the texture (Kruskal-Wallis statistic= 4.087, $p=0.252$ (table 3).

Regarding *S. enteritidis*, there were no statistically significant variations among the different treatments according to the pH of the samples ($F= 0.99$, $p=0.411$). There were no statistically variations among the different treatments according to the texture (Kruskal-Wallis value = 3.231, $p=0.357$), the odor of the samples (3.591, $p=0.309$), the color of the samples (1.091, $p=0.779$), and the overall acceptability (0.352, $p=0.95$) (table 3).

Table 3. Sensory attributes of control and treated beef samples contaminated with *S. virchow* and *S. enteritidis*.

| <i>Salmonella</i> spp. | Experiment Group | pH | Texture | Odor | Color | OAA |
|------------------------|------------------|-------------|------------|------------|------------|------------|
| <i>S. virchow</i> | Control | 5.20 ±0.131 | 4.3 ±0.823 | 3.9 ±0.738 | 3.9 ±0.738 | 3.9 ±0.568 |
| | SAEW | 5.20±0.076 | 3.8 ±0.789 | 4.3 ±0.823 | 4.2 ±0.789 | 4.1 ±0.876 |
| | 1%FA | 5.25±0.151 | 3.6 ±0.699 | 4.3 ±0.823 | 4.4 ±0.699 | 4.4 ±0.699 |
| | SAEW+FA | 5.25±0.151 | 3.8 ±0.789 | 4.3 ±0.823 | 4.3 ±0.823 | 4.2 ±0.789 |
| <i>S. enteritidis</i> | Control | 5.16 ±0.106 | 4.1 ±0.876 | 4 ±0.667 | 4.1 ±0.738 | 4.2 ±0.632 |
| | SAEW | 5.24±0.119 | 4 ±0.816 | 4.5 ±0.707 | 4.4 ±0.843 | 4.1 ±0.876 |
| | 1%FA | 5.26±0.141 | 3.6 ±0.699 | 4.5 ±0.707 | 4.2 ±0.789 | 4.3 ±0.823 |
| | SAEW+FA | 5.25±0.141 | 4.2 ±0.789 | 4.3 ±0.823 | 4.3 ±0.823 | 4.2 ±0.789 |

DISCUSSION

Food safety threats posed by food-borne infections, such as *Salmonella*, continue to be of great concern for the industry of food. Diseases caused by *Salmonella* are major public health issues and more important worldwide in developing countries (Wang et al. 2008).

Many sanitizers, as SAEW, FA, hydrogen peroxide, chlorine dioxide, and sodium hypochlorite have been widely employed for their efficacy in decreasing or eliminating pathogenic microorganisms in food manufacturing (Koide et al. 2011).

SAEW has an effective bactericidal action based on its low pH, high ORP values, and high ACC values. Huang et al. (Huang YuRu et al. 2008) concluded that ACC has substantial role in the bactericidal impacts of SAEW more than other factors as high ORP and low pH value. ACC values are associated with available chlorine which contributes primarily to HOCl, which is a potent antimicrobial substance (Cao et al. 2009). HOCl penetrates microbial cell walls, disrupting proteins, DNA, and enzymes, leading to cell death. The high ORP of SAEW enhances its capability to destroy bacterial cell membranes and denature critical cellular components. The acidic nature of SAEW can destabilize the bacterial cell wall and membrane, increasing the efficacy of its oxidative agents (Meghwar et al. 2024).

In this study, the use of SAEW led to a log reduction of 1.94 log cfu/g (30.9%) and 2.15 log cfu/g (33.3%) in *S. virchow* and *S. enteritidis*. Higher data were gained by (Bing et al. 2022) who mentioned that treatment of beef samples with SAEW results in a log reduction of 3.36 log CFU/g in case of *S. enteritidis*. Similar results were reported by (Rahman et al. 2012), (Al-Holy and Rasco 2015), and (Mansur et al. 2015) who reported a log reduction of 2.99, 2.3, and 1.6 log CFU/g in *S. typhimurium* when treated with SAEW.

Sanitization effectiveness of SAEW has been confirmed in eliminating *Salmonella* on a variety of foods, including fresh vegetables and fruits as well as meats; beef and pork (Kim, Hung, and Russell 2005), poultry and eggs (Fabrizio et al. 2002), and seafood (Ozer and Demirci 2006). SAEW has also demonstrated as a surface disinfectant for numerous food processing utensils (Deza, Araujo, and Garrido 2005).

Fumaric acid has antimicrobial effects against *Salmonella* spp. The undissociated form of fumaric acid can cross the cell membrane of bacteria, disrupt intracellular pH and metabolic functions. Also, it can interfere with bacterial energy metabolism by targeting enzymes or pathways crucial for *Salmonella* survival and growth (ABDEL-WAHHAB et al. 2023).

Through the current study, the use of 1% FA led to a log reduction of 1.69 log cfu/g (26.9%) and 1.83 log cfu/g (28.5%) in *S. virchow* and *S. enteritidis* respectively. Many reports confirmed the effectiveness of using FA for minimizing *Salmonella* on fresh beef surfaces. Many studies; **Podolak et al. (1995, 1996)** mentioned the reduction of this microbe was less than 2 log CFU/g and that supported by **(Fabrizio and Cutter 2004; Podolak et al. 1995, 1996)** and **(Rahman, Wang, and Oh 2013)**.

In the present work, the combination of 1% FA and SAEW 30 mg/L resulted in a log reduction of 3.14 log cfu/g (48.7%) and 2.93 log cfu/g (46.7%) in *S. enteritidis* and *S. virchow*. Numerous studies reported that the incorporation of SAEW 30 mg/L and 0.5% FA was associated with greater decreases of pathogens paralleled to control and all sole treatments ($P < 0.05$), which minimized *S. typhimurium* by 2.6 and 2.99 log CFU/g **((Mansur et al. 2015)**.

The synergetic bactericidal impact of SAEW and FA might be attributed to the enhanced physicochemical characteristics of both sanitizers. The addition of FA to SAEW decreases the ACC and pH values of SAEW and increases its ORP value. The integrated action of ACC, chlorine compound, pH, and stable molecules of 1% FA and SAEW can explain their boosted bactericidal efficacy in the minimizing of pathogens in fresh beef. Undissociated forms of FA may act as a permeabilizer of the cell membrane of bacteria and react as a potentiator of the impacts of SAEW to boost its antimicrobial action. The sequential antimicrobial application of SAEW and 1% FA was more efficient in the reduction of microbial contamination in meat fatty tissue than sole sanitizing treatments **(Pohlman et al. 2002)**. Furthermore, the lower chlorine concentration in the combined treatment enhances its safety and makes it more suitable for meat industry applications. **Byelashov and Sofos (2009)** have approved chlorine conc. at 20 mg/L for poultry sprays and washes in the United States.

In general, the application of sanitizer treatments can decrease the bacterial contamination

load but does not completely eradicate pathogens. Multiple causes that can influence microbial responses to disinfection include initial microbial load, treatment conditions, low-nutrient growth, adherence to surfaces, aggregation and encapsulation. Increased resistance to disinfection can be attributed to the adherence or association of microorganisms to diverse particulate surfaces. Therefore, before the selection and application of a food sanitizing technique, it is crucial to know the kind of organism being goaled and its linked resistance to the selected technique **(Sun et al. 2022)**.

*The present study revealed that *S. virchow* is more resistant to treatment by sanitizers than *S. enteritidis*.* **(Yoon et al. 2017)** mentioned that *S. virchow* is generally considered **more virulent** than *S. enteritidis* due to its **higher invasiveness, and antibiotic resistance**.

In the present study, no noticeable changes in the OAA, odor, and pH of beef specimens inoculated with *S. virchow* or *S. enteritidis* after treatment with sanitizing agents in comparison to the control. Groups treated with sanitizing solutions presented a more clearly intense pinker color than the control group. Regarding the texture profile FA treatments caused a slight increase in firmness and work of shear. Similar data were obtained by **(Sheng et al. 2018)**, **(Naka et al. 2020)**, **(Biswas et al. 2024)** who demonstrated that SAEW has no effect on the sensory properties of beef samples.

(Rahman et al. 2012) reported that SAEW treatment can improve sensory properties. It contains HOCl and -OH that have potent antimicrobial and antioxidant effects that help to keep oxidation stability with SAEW-washed meat specimens better than control ones. Furthermore, the residual hydrochloric acid content of SAEW (although minute) preserves meat odor and gives meat specimens a fresh color.

Fumaric acid helps stabilize myoglobin, which responsible for meat's red color enhances the formation of oxymyoglobin by reducing oxidation and lowering pH, it slows down discoloration and helps retain the bright red color

longer as mentioned by **Hecer and Guldas (2011)**, (**Gómez et al. 2020**) and (**Shi et al. 2022**), and (**Vila-Clarà et al. 2024**), (Hecer and Guldas 2011) reported that no color loss and no impact was seen on odor obtained with the application of 1% FA after slaughtering. (**Fernández et al. 2021**) reported that FA lowers pH, which can cause protein denaturation and result in a firmer texture.

CONCLUSION

None of the tested antimicrobial agents completely eliminated *Salmonella* contamination (serovars enteritidis and virchow). Reduction rates varied between the two agents evaluated (electrolyzed water and fumaric acid). Further research is needed under a range of conditions and with additional *Salmonella* strains to investigate the influence of genetic variations on antimicrobial resistance. This should include exploring higher concentrations of the tested disinfectants, evaluating alternative disinfectants, or investigating synergistic effects by combining them with other antimicrobial agents. Crucially, any such agents must demonstrate potent antimicrobial activity while ensuring consumer safety and minimizing environmental impact.

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