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Efficacy of Electrolyzed water in controlling microbial contamination of fresh chicken carcasses

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ABSTRACT

As an increasing international need for natural sanitizers and disinfectants especially in food facilities, instead of the wide use of chemical sanitizers and disinfectants, that is because of their known disadvantages as their toxic residues in food and their bad effect on nature. There have been many studies done on Electrolyzed water (EW), as a novel broad-spectrum disinfectant and cleaner, which has been widely used for several years. EW can be produced in an electrolysis chamber containing dilute salt and tap water. It is an effective antimicrobial and antibiofilm agent, with several advantages such as being on-the-spot, cheap, environmentally friendly, and safe for human beings. The routine in most chicken slaughtering houses is using chlorine in the last wash before selling fresh refrigerated chicken. So, the present study was conducted to evaluate the beneficial effects of replacing chlorine with slightly acidic electrolyzed water (SAEW) with PH (4-6) freshly prepared in the lab on 32 fresh chicken carcasses samples. The samples were tested for Total bacterial count, Enterobacteriaceae count, and detection of E. coli in these samples. The results were promising and the effect of SAEW showed decreasing in the total bacterial count, Enterobacteriaceae, and total E. coli count by mean reduction percentages of 88.2%, 85.3%, and 98.4% respectively.

INTRODUCTION

The concept of safe and wholesome food from a food safety aspect, is food that is free not only from toxins, pesticides, chemicals, and physical contaminants but also from microbiological pathogens that can cause food born disease **(Roberts 2001).**

Chicken provides us with animal protein of high biological value essential at all ages as it contains all the essential amino acids required for growth with a high proportion of unsaturated fatty acids and low cholesterol value. Moreover, poultry meat is a good source of different types of vitamins such as niacin, riboflavin, thiamine, and ascorbic acid as well as sodium, calcium, iron, phosphorus, sulphur, and iodine **(Abou Hussein 2007)**, but also it represents a favourable media for growing all kind of microorganisms, at this time arises the necessity

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of keeping this product clean, fresh and increase their shelf life in many ways.

The last stage of production before introducing the refrigerated chicken to the markets is the last wash before backing, this step is very important to ensure the cleanness of the product with the minimum superficial bacterial load. The usual routine in the poultry slaughtering house is to immerse the chicken carcasses in a water tank with a percentage of chlorine or spray them with water spray with additional chlorine to decrease the superficial bacterial load increase the product quality and increase its shelf life as well **(FAO, 2015).**

Hypochlorite, one of the products most widely used chlorine disinfectants, has a broad spectrum antimicrobial activity, does not leave toxic residues, not affected by water hardness, inexpensive, as it fast-acting removes dried or fixed organisms and biofilms from surfaces, but on the other hand, it can produce ocular irritation or oropharyngeal, oesophageal, gastric burns and may leave an undesirable odour on the final product if the chlorine percentage is more than the permissible limit **(HSA, 2023).**

On the other hand, Electrolyzed water (EW) is a novel disinfectant and cleanser which has been widely used in the food industry for several years to ensure the sterilization of surfaces and the safety of food **(Arya 2018).**

(USEPA) US Environmental Protection Agency recommended the use of disinfectants with hypochlorite acid as active ingredients for the disinfection of surfaces against COVID-19 **(Samara 2020).**

EW is produced in an electrolysis chamber that contains dilute solution of tap water and sodium chloride salt without any harmful chemical additives. EW has antimicrobial effects against a variety of microorganisms including common biofilm, viruses, bacteria, spores, and fungi in chronic wounds and environmental surfaces **(Lemos 2020).**

Currently, due to its beneficial anti-infection and cell proliferative properties, researchers pay more attention to the application of electrolyzed water in clinical treatments including medical sterilization.

The wide spread of many dangers diseases has brought heightened attention to the importance of cleaning, sanitizing, and disinfecting in retail food and food service establishments. In response, major governmental agencies have emphasized the need to frequently disinfect high-touch surfaces. In the retail food and food service industry, sanitization is a routine, common practice defined and recommended in the U.S. Food and Drug Administration (FDA) Food Code **(Angela et al. 2021).**

Electrolyzed water (EW) is emerging as an environmentally friendly safe antimicrobial treatment **(Huang et al. 2007).**

National Institutes of Health (NIH) reported that biofilms are responsible for up to 80% of human bacterial infections **(Jamal et al. 2018).** So, if we can reduce or get rid of this biofilm with a safe disinfectant such as EW and it will reflect on human health very well.

After poultry slaughtering and cleaning, as a regular protocol, poultry carcasses are sprayed or immersed in water with added chlorine with a certain percentage to reduce the surface bacterial load in order to improve their quality and increase the shelf life in the refrigerator.

So, this study aimed to illustrate the effect of slightly acidic electrolyzed water with pH (4 -6) on fresh chicken carcasses by immersing them for five minutes and show this effect on a total bacterial count, total Enterobacteriaceae count, and total E. coli count of the examined samples.

MATERIAL AND METHODS

1. Samples collection: 32 random samples of freshly slaughtered chickens were collected from many poultry retail shops. They were collected just after slaughtering and washing only with tap water without any additives and were transferred to the lab in an ice box as soon as possible.

2. Preparation of samples: was carried out according to **(ISO 2003)**.

3. Aerobic Plate Count: was carried out according to **(ISO 2013)**.

4. Enterobacteriaceae count: was carried out according to **(ISO 2004)**.
5. Isolation and identification of Enteropathogenic *E. coli*: was carried out according to **(Quinn et al. 2002)**.

6– Slightly acidic electrolyzed water (SAEW) was prepared in the lab by preparing a simple apparatus consisting of two chambers each one containing tab water with 2 gm. NaCl salt for each litre of water, and in this water an electric current was used from 400 wat adaptor the test was repeated three times.

7- Each time the samples were divided in tow groups, one was not treated with EW (pre-treatment group) the other group was treated by immersing in EW for five minutes.

Statistical analysis

The data collected were subjected to analysis by t-independent test and Fischer Exact Probability test using **SAS (2004)** software, the data was considered significant at P <0.05.

RESULT

Table 1. Total bacterial count analysis for 32 fresh chicken carcasses before and after immersing in SAEW, the test was repeated three times

Group	Mean	SE	Minimum	Maximum
Control	7.6×10^4	3.4×10^5	2.6×10^2	10×10^5
Treated	6.1×10^4*	1.5×10^4	4.6×10	3×10^4

* Significant difference using Fischer Exact Probability test at P < 0.05.

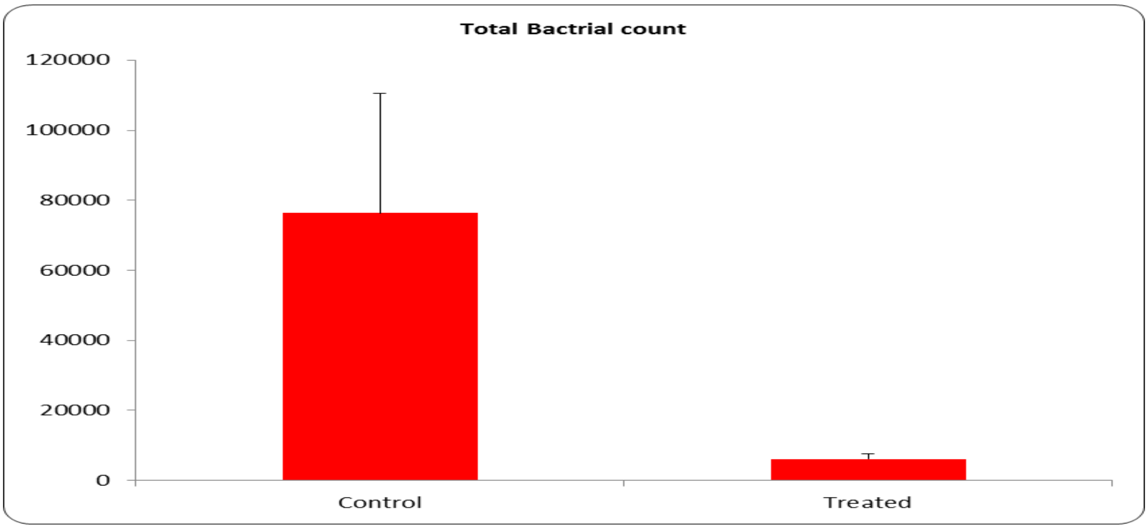

Figure (1) Total bacterial count analysis for 32 fresh chicken carcasses before and after immersing in SAEW, the test was repeated three times.

Table 2. Total Enterobacteriaceae count analysis for 32 fresh chicken carcasses before and after immersing in SAEW, the test repeated three times.

Group	Mean	SE	Minimum	Maximum
Control	8.1×10^3	5.2×10^3	1.5 x10	10×10^4
Treated	4.3×10^2*	9.1 x 10	10	1.4×10^3

* Significant difference using Fischer Exact Probability test at $P < 0.05$.

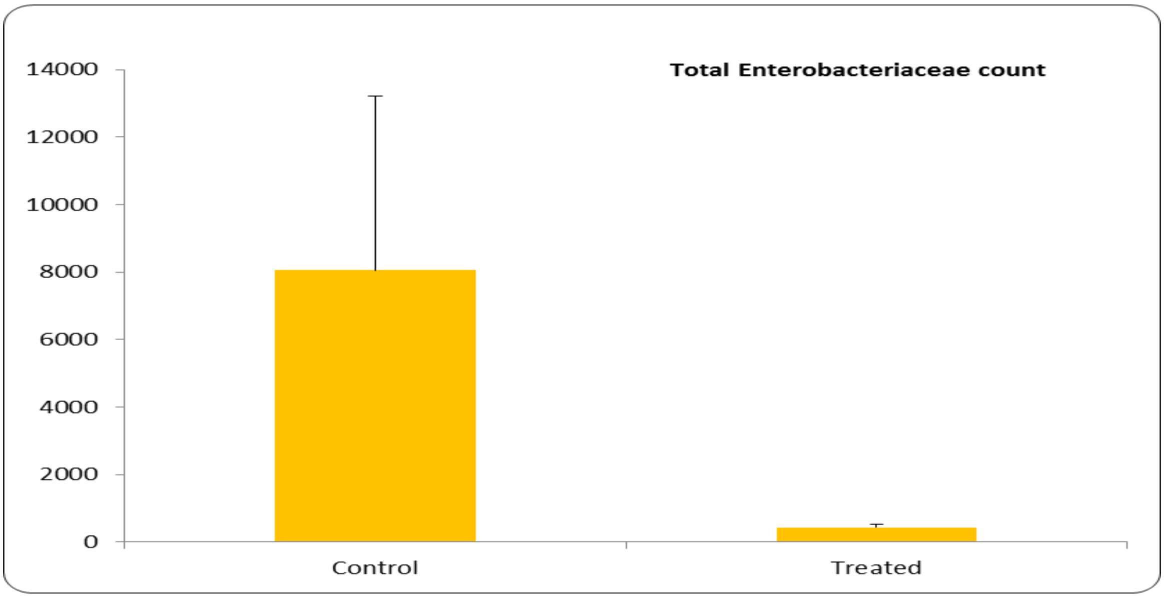

Figure (2) Total Enterobacteriaceae count analysis for 32 fresh chicken carcasses before and after immersing in SAEW, the test repeated three times.

Table 3. Incidence of pathogenic E. coli for 32 fresh chicken carcasses before and after immersing in SAEW, the test repeated three times.

Group	Incidence of pathogenic E. coli
Control	21.9 %
Treated	6.25%

Table 4. Total count of pathogenic E. coli for 32 fresh chicken carcasses before and after immersing in SAEW, the test repeated three times.

Group	Mean	SE	Minimum	Maximum	Significance
Control	1.8×10^2	4.5 x 10	1×10^2	4×10^2	T value = 1.69 P value=0.1350
Treated	3.0 x 10	2.0 x 10	10	5.0 x 10	

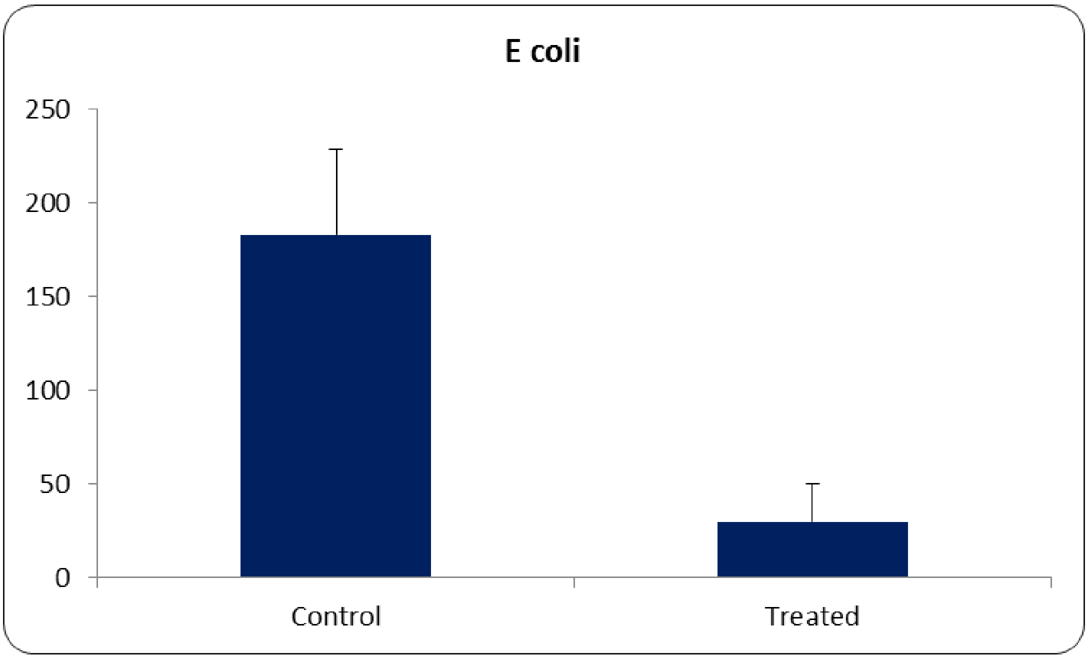

Figure (3) Count of pathogenic E.coli for 32 fresh chicken carcasses before and after immersing in SAEW, the test repeated three times.

Table 5. Sensory changes / Time for 64 fresh chicken carcasses before and after immersing in SAEW, to get deteriorated and become unfit for human consumption the test was repeated three times.

	Control	**1est group**	**2end group**	**3rd group**
1est-6th day	No change	No change	No change	No change
7th day	Bad smell and discoloration	No change	No change	No change
9th day	-	No change	Bad smell and discoloration	No change
10th day	-	No change	-	No change
11th day	-	Bad smell and discoloration	-	Bad smell and discoloration

Just after the bad smell and the discoloration appeared samples were culled safely.

Table 6. Time for 32 fresh chicken carcasses before and after immersing in SAEW, to get deteriorated and unfit for human consumption the test was repeated three times.

Group	**Mean**
Control	7.75 ± 0.0778
Treated	9.25 ± 0.1489 *

Data represented as mean ± SE
* Significant as mean ± slandered error
* Significant at $P < 0.001$ using t-student test.

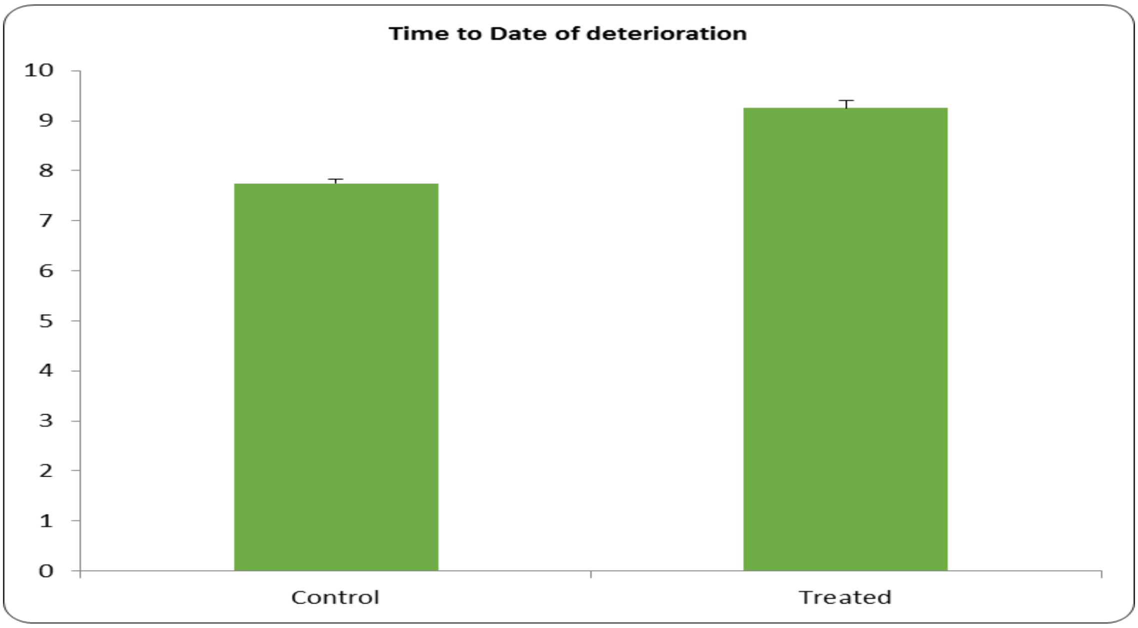

Table 7. the reduction percentage in different bacteriological tests

Bacteriological test	The mean value of Reduction %
Total bacterial count	88.2%
Total Enterobacteriaceae count	85.3%
Total E. coli count	98.4%

Reduction Equation = (Initial load – New load) /Initial load x 100

DISCUSSION

water used in homes, businesses, and industries is often treated through a chemical process to remove harmful substances and bacteria. Once water has been chemically treated, it can be safely recycled back into the water source. Several different chemicals can be used to treat water, such as chlorine, lime, and hydrogen peroxide. Each method offers distinct advantages and disadvantages for use in water treatment **(Sara Melone 2017).**

Chlorine chemistry helps provide safe and abundant food by protecting crops from pests and keeping kitchen counters and other food-contact surfaces disinfected, decreasing foodborne diseases by destroying *E. coli, Salmonella,* and a host of other foodborne germs **(Hayashibara 1994)**.

Researchers have used electrolyzed technology to produce multi-functional EW. EW shows many kinds of benefits. Acidic EW effectively suppresses many harmful bacteria and has also been used as a new type of disinfectant (containing HOCl) and cleaning agent (containing NaOH) in recent years **(Rahman et al.**

2016).

EW is also used in combination with other methods, such as organic acids, ultrasound, and mild heating **(Liu & Chen 2020)** to perform more effective sterilization.

Acidic Electrolyzed water (AEW) is more effective as a disinfectant. AEW is mostly used as drinking water to promote health benefits and prevent some diseases **(Higashimura et al. 2018)**.

Electrolyzed water (EW) is produced in an electrolysis chamber that contains hydrogen chloride (HCl) solution or dilute salt (NaCl) **(Rahman 2010).**

It is produced by electrolysis of a dilute salt solution, and the reaction products include sodium hydroxide (NaOH) and hypochlorous acid **(Huang et al. 2007)**.

Three forms of the solution can be produced, an acidic form, a neutral pH form, and an alkaline form. AEW exhibits an acid pH, a high oxidation-reduction potential, and high free chlorine concentrations which makes it effective as an antimicrobial agent **(Xuan et al. 2019)**.

Electrolyzed water applications in different sections have already proved their potential for being used as one of useful sanitizers in the food, aquaculture, agriculture, medical, and energy industry. Recently, many start-up companies and industries started commercialization and marketing different types of electrolyzed water all around the world **(Setareh et al. 2018).**

Moreover, the top five food categories linked to food poisoning outbreaks in the USA from 1990 to 2003 were seafood, dairy products, eggs, beef, and poultry products which were responsible for 61% of all outbreaks according to the Centre for Science in the Public Interest (CSPI)'s database **(Cichoski et al. 2019).**

In this study (SAEW) slightly acidic electrolyzed water was prepared in the lab by preparing a simple apparatus as shown in this figure (1) consisting of two chambers each one containing tab water with 2 gm. NaCl salt for each litre of water, and in this water an electric current was used from 400 wat adaptor as shown in Figure (3) the test was repeated three times. The water pH was (4 -6) slightly acidic as shown in Figure (2) and the pH meter was used to measure this pH and the time of immersing was five min.

Figure (1)

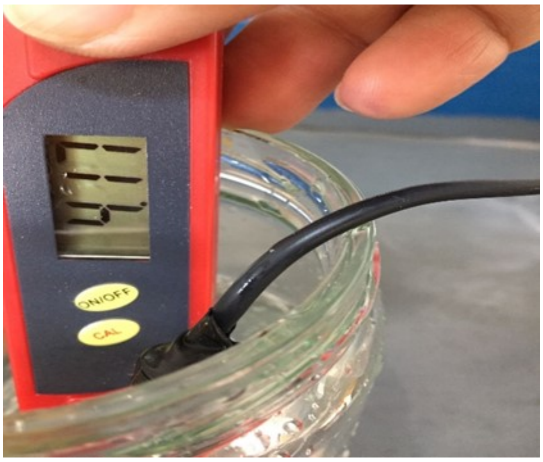

Figure (2)

Figure (3)

Each time the samples were tested by surface swabs for Total bacterial count, Enterobacteriaceae count and E. coli count.

Aerobic plate count can provide a general indication of the microbiological quality of food **(NSW/FA 2009).**

Potential Food Safety Hazard top aerobic plate count (APC)which **indicates the level of microorganisms in a product. Aerobic plate counts** sometimes can be useful to indicate quality, shelf life, and post-heat-processing contamination **(*Corrosionpedia*, 2019)**.

So, in this experiment, the test revealed that the mean value of total bacterial count decreased from 7.6 x 10^4 to 6.1 x 10^4, after treatment by immersing fresh chicken carcass samples in SAEW for 5 min. by reduction rate 88.2% .

That decrease in the superficial bacterial load will improve the quality of the sample and increase their shelf life which actually increased in this experiment from 7.75 days to 9.25 days in their mean.

Enterobacteriaceae are a large family of bacteria that can be found in many environments, including food. They are used as indicators of hygiene, sanitation, and post-processing contamination of heat-processed foods. They can also cause foodborne illness and diarrhoeal diseases Some Enterobacteriaceae are part of the normal gut flora, while others are pathogenic **(Chris Baylis 2011).**

Members of the family are responsible for causing foodborne disease and some also cause food spoilage and therefore contribute to substantial economic losses and food wastage **(FSAI 2016).**

The mean value of Enterobacteriaceae count decreased from 8.1 x 10^3 before the treatment of the sample to 4.3 x 10^2 after immersing the samples in SAEW by reduction rate 85.3 %, which proves the antibacterial effect of the electrolyzed water.

So, if this protocol is used in chicken slaughtering houses as it shown in figure (4) it will not only decrease the bacterial load and increase the shelf life but also it will affect the pathogenic microorganisms and decrease the hazard of food-borne diseases.

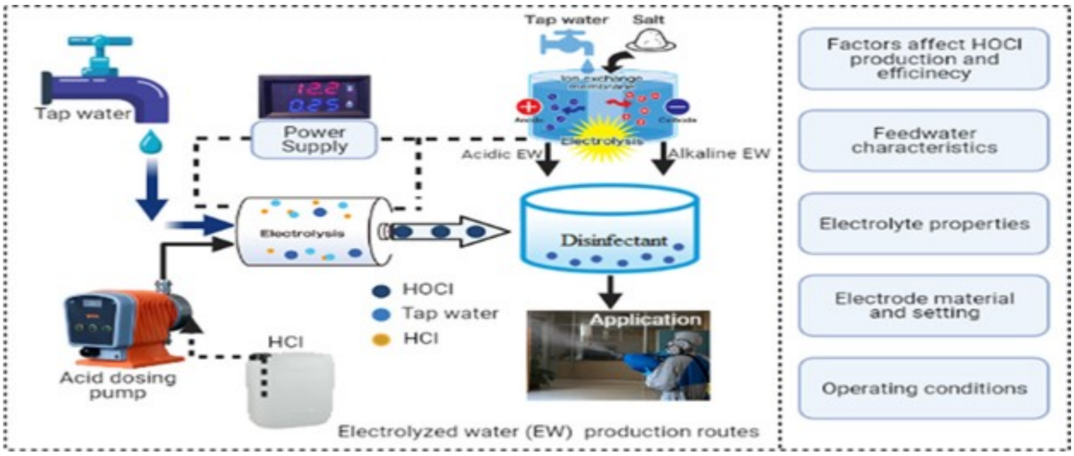

figure (4)

Moreover, the mean value of pathogenic E. coli count decreased from 1.8 x 10^2 to 3.0 x 10 after sample treatment by a 98.4 % reduction rate.

Also, the incidence of E. coli was 21.9%in pre-treatment samples' and was decreased to 6.25 % after treatment with EW.

However, under a poor hygienic environment, raw chicken meat presents an ideal substrate supporting the growth of pathogenic ***Escherichia coli* and Coliform bacteria** indicating the potential presence of other pathogenic bacteria; this may constitute a major source of food-borne illnesses in humans **(Joyce Arua Odwar et al. 2014).**

Enteropathogenic *Escherichia coli* strains are the major cause of many infantile diarrhea, in typical cases; symptoms appear within 12 to 36 hours. Clinically, EPEC illness is characterized by fever, nausea, vomiting, and watery stools, which occasionally contain mucous, but without gross blood (**Medina-Gudiño et al. 2020**).

The mean time needed for the untreated samples to deteriorate and become unfit for human consumption was 7.75 days, while for the treated samples with SAEW, it was 9.25 days. So, the antimicrobial and antibiofilm effect of SAEW not only decrease the superficial bacterial load and decrease the hazard of food-borne disease but also increases the shelf life of these samples which may have economic benefit as well.

In comparing the inhibitory effects of AEW (acidic electrolyzed water) and sterile deionized water containing free chlorine on pathogenic bacteria, the results reveal reductions in the bacterial counts of both pathogens similar to those observed with AEW (acidic electrolyzed water) **(Venkitanarayanan et al. 1999)**.

In subsequent studies, the antibacterial effect of AEW was proved repeatedly **(Park et al. 2009)**. In addition, Researchers have found EW able to control *E. coli* O157:H7 on various vegetables **(Hui-Fang et al. 2011).** EW has been proposed as an environmentally friendly alternative to physical and chemical methods, which do not contain undesirable toxic contaminants (**Feng P. et al. 2002).**

Medina- Gudiño et al. (**2020)** reported that artificially contaminated eggs with *Salmonella* or *E. coli* reduced >1.45 Log10 CFU/egg and > 6.39 Log10 CFU/egg, respectively, after 30 seconds treatment of NEW. In the United States, EW is allowed on beef carcasses applied as a spray at a level not to exceed 50 ppm calculated as free available chlorine **(USDA/ FSIS, 2014 and Vijay 2021).**

CONCLUSIONS

EW is an effective disinfectant, with several advantages such as on-the-spot, cheap, environmentally friendly, and safe production.

Nowadays, with the development of a novel popular type of SAEW, some limitations have been resolved. It has been reported that SAEW does not irritate the hands, skin, and mucous membranes, and causes no safety issues from Cl_2 off-gassing.

It recently emerged with great potential for clinical applications. However, the antimicrobial effect of EW is influenced by the presence of organic matter, water pollutants, and the hardness of the product.

Therefore, a dynamic and advanced EW production system or the hurdle technology of combing with multiple technologies-based EW can overcome current limitations. These may expand the use of EW in clinical applications.

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