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Impact of slightly acidic and alkaline electrolyzed water on shelf-life of the chilled chicken fillet

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ABSTRACT

Electrolyzed Water is a potent multifunctional antibacterial agent with numerous applications in food safety via processing, storage as well as it has great importance in agriculture and medicinal applications. The current study investigate the powerful of the sanitizing effect of slightly acidic (SAcEW) as well as the detergent effect of slightly alkaline electrolyzed water (SAlEW) (individually and in combination) on chilled chicken fillets subjected to a dipping treatment for 5 min and stored refrigerated at 4±1°C. When treated were compared to untreated samples, it was turned out to be that SAlEW and SAcEW treatments increased the shelf life of chilled chicken fillet and remained fit for consumption till 7th and 9th days, recorded APC 4.89±0.01 and 4.9±0.0, psychrophilic plate count 3.8±0.02 and 2.18±0.03, *E. coli* count 1.97±0.03 and 1.98±0.02 and Molds count 3.84±0.02 and 3.56±0.68, respectively. Moreover, dipping of chicken fillet in SAlEW followed by SAcEW resulted in prolong the shelf-life until the 11th day of storage, recorded APC 4.94±0.01, psychrophilic plate count 4.67±0.02, *E. coli* count 1.98±0.03 and Molds count 3.88±0.03. All treatments were effective in reducing microbial populations throughout the storage, with combined treatment showing the strongest antimicrobial activity. So, EW can be effectively used to eliminate or at least to reduce bacterial contamination to an acceptable Food safety limits listed in national and international standards, references and recognized food constitutions that considered being safe for human consumption and subsequently, extending shelf life of chilled foods including chicken fillet during processing, distribution, storage and marketing.

INTRODUCTION

In Egypt, chicken is a very popular food source, because chicken meat is highly perishable, proper storage is essential, that aids chicken meat products not be exposed to contamination with different microorganisms, while stor-

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ing and marketing **(Hong et al. 2008a;b)**.

Various techniques for reducing bacterial contaminations have been used to improve the microbial safety of chicken meat **(González-Fandos and Dominguez, 2007; Kim and Day, 2007 and Hyeon et al. 2013)**.Various sanitizing processes have recently been implemented to improve the safety and quality of fresh meat and meat products prior to refrigeration **(Awad et al. 2012 and Guan and Fan, 2010).** Furthermore, consumers are concerned about the use of chemicals as a sanitizing agent, because they may have harmful effects on human health. As a result, most studies on the decontamination of fresh meat or vegetables have focused on sanitizing agents other than chemical sanitizers, as chlorine, per acetic acid, and hydrogen peroxide **(Guentzel et al. 2008; Gil et al. 2009; De São José and Vanetti, 2015 and Mansur and Oh, 2015).** Electrolyzed Water (EW) has been used as a disinfectant and sanitizing agent in the food industry in USA and Japan, as an antimicrobial treatment method that has recently gained interest due to its confirmed applications in the food industry. It has also been demonstrated that the action of EW in the suspensions is greater on the food and equipment surfaces **(Huang et al. 2008 and Attia et al. 2021).**

Three types of electrolyzed water (Acidic, Neutral and Alkaline) are currently being investigated for their efficacy as antimicrobial agents in biological systems. Pathogenic agents such as bacteria, fungi, viruses, protozoa, algae, and nematodes are among the many applications of electrolyzed water **(Al-Haq et al. 2005).**

Therefore, the goal of this study was to determine the impact of SAcEW, SAlEW, and a combination of two affected the shelf life and the microbial growth of fresh chicken fillet stored at 4±1°C.

3- MATERIAL AND METHODS

3-1 Sample collection and preparation

A total of 500 gram of fresh chicken fillet was purchased from the same shop of Cairo market – Egypt, transferred to the laboratory as soon as possible under strict hygienic measures to carry out the experiment. The sample mass was divided into five groups (100 g each); 1st group was kept in its original container without rinse, the 2nd rinsed with DW, 3rd was rinsed with SAlEW, 4th was rinsed with SAcEW and the 5th group was rinsed with SAlEW followed by SAcEW for five minutes each. All samples were drained, packed in polyethylene bags and stored at 4±1°C and examined at zero time, 1st, 3rd, 5th, 7th, 9th, 10th, 11th and 12th days of storage or when APC exceeding the permissible limit according to the Egyptian standard (**ES 1651, 2019**). The experiment was repeated in triplicate.

3.2. Aerobic plate count (APC) according to APHA (2001)

Ten grams of each sample was weighed out under aseptic conditions and placed in sterile "Stomacher" bags for the microbiological analysis. Then, 90 ml of sterile physiological saline was added and homogenized for two minutes. Ten-fold serial dilutions were prepared. The total bacterial count was determined on agar medium using Standard Plate Count Agar (Oxoid, CM0463). Incubation was run at 35°C ±1°C for 48 h. Bacterial counts were given in $\log_{10}$ cfu/g.

3.3. Total Psychotropic count (APHA, 2001): -

Psychotropic count was determined in a similar method to that for APC, except that plates were incubated at 7±1°C for 10 days. The colonies were counted and expressed as $\log_{10}$cfu/g of sample.

3.4. Enumeration, Isolation and identification of β-glucuronidase-positive *Escherichia coli* according to ISO (16649-2:2001) (TBX method):

This method for enumeration and isolation of B-glucuronidase – positive *Escherichia coli* which grow at 44°C form typical blue colony on tryptone –bile-glucuronide medium (TBX) in all kinds of food and feed of animal origin.

Enumeration of mold and yeast according to ISO 21527/1 (2008)

From each dilution, 0.2 mL. of each dilution was transferred to DG18 dechlorane rose Bengal agar plates, distributed by sterile glass spreader, plates were incubated at 25°C±1°C for 5 to 7 days, counts were recorded as $\log_{10}$cfu/g sample

Preparation of Slightly acidic and alkaline electrolyzed water (SAcEW and SAlEW) according to Hricova *et al.* (2008) and Athayde *et al.* (2018) as follows: -

Sufficient amount of potable drinking water was prepared with addition and dissolving of sodium chloride (NaCl) by a rate of 2 g for each 1 liter of water (0.2 %).

A current of 9-10 volt-amber (VA) was passed through the water using an electrolysis cell with two poles of anode (+) and cathode (-). Upon the onset of the electrolysis process, NaCl was dissociated into Na^+ and Cl^-

Meanwhile, water was reduced at the cathode pole formed hydroxide (OH^-) and Hydrogen (H^+) ions in the solution according to the following formula: $2H_2O + 2e^- \longrightarrow H_2^+ + 2OH^-$

Negatively charged ions represented by the hydroxyl group (OH^- and Cl^- move towards the anode where electrons are released and hypochlorous acid (HOCl), hypochlorite ions (^-OCl), oxygen gas (O_2) and chlorine gas (Cl_2) and HCl were generated.

Positively charged ions (Na^+ and H^+) move toward the cathode where they gain electrons, resulted in the generation of sodium hydroxide (NaOH) and hydrogen gas (H_2).

A few drops of vinegar 5%, if necessary, were added to the electrolyzed water to adjust the pH 5.5 to be slightly acidic (SAEW).

Statistical analysis

Statistical analysis were run in triplicate and results were reported as mean values and standard deviation (Mean±SD) using of Statistical Packaging for the Social Science (SPSS) Ver. 20. A p-value less than 0.05 ($p \leq 0.05$) was considered statistically significant.

RESULTS

Table 1. Effect of different types of rinsing water on the hygienic status and the shelf-life of chicken fillet

Type of water treat-ment/ count	Storage time by days								
	Zero time	1st	3rd	5th	7th	9th	10th	11th	12th
APC									
Non water treatment	4.0±0.02	4.8±0.01	4.88±0.04	4.95±0.1	5.62±0.03				
Using DW	3.9±0.01	4.0±0.07	4.75±0.13	4.9±0.01	5.5±0.02				
SAlEW	3.3±0.07	3.55±0.03	4.25±0.05	4.56±0.02	4.89±0.01	5.35±0.06			
SAcEW	3.17±0.04	3.24±0.01	3.72±0.01	3.89±0.02	4.3±0.03	4.9±0.01	5.70±0.01		
SAlEW+ SAcEW	2.92±0.01	3.18±0.01	3.24±0.01	3.61±0.01	3.86±0.01	4.14±0.12	4.78±0.04	4.94±0.01	5.78±0.07
Psychotropic count									
Non water treatment	<1	<1	4.30±0.04	4.63±0.11	4.70±0.12				
Using DW	<1	<1	3.80±0.01	4.40±0.01	4.60±0.02				
SAlEW	<1	<1	<1	2.0±0.04	3.8±0.02	4.9±0.02			
SAcEW	<1	<1	<1	<1	<1	2.18±0.03	3.90±0.01		
SAlEW+ SAcEW	<1	<1	<1	<1	<1	2.11±0.01	3.7±0.02	4.67±0.02	5.23±0.01
E. coli count									
Non water treatment	1.95±0.01	1.96±0.01	1.97±0.01	1.99±0.01	2.16±0.01				
Using DW	1.91±0.01	1.92±0.02	1.95±0.04	1.98±0.01	2.03±0.05				
SAlEW	1.83±0.02	1.91±0.02	1.93±0.01	1.95±0.01	1.97±0.03	2.01±0.05			
SAcEW	1.07±0.04	1.77±0.02	1.83±0.05	1.90±0.01	1.95±0.01	1.98±0.02	2.08±0.04		
SAlEW+ SAcEW	1.48±0.03	1.59±0.01	1.67±0.03	1.71±0.02	1.80±0.02	1.87±0.02	1.93±0.03	1.98±0.03	2.06±0.08
Mold count									
Non water treatment	2.72±0.01	2.83±0.06	3.30±0.04	4.51±0.02	5.1±0.03				
Using DW	2.23±0.02	2.82±0.07	3.54±0.03	4.06±0.05	4.78±0.02				
SAlEW	2.22±0.02	2.69±0.05	2.92±0.01	3.18±0.01	3.84±0.02	4.62±0.01			
SAcEW	2.19±0.04	2.61±0.02	2.78±0.01	3.07±0.04	3.64±0.04	3.56±0.68	4.65±0.05		
SAlEW + SAcEW	1.97±0.07	2.21±0.02	2.53±0.04	2.72±0.07	2.91±0.01	3.09±0.03	3.55±0.03	3.88±0.03	5.08±0.59

All microbial counts were expressed as mean $\log_{10}$ cfu/g±SD

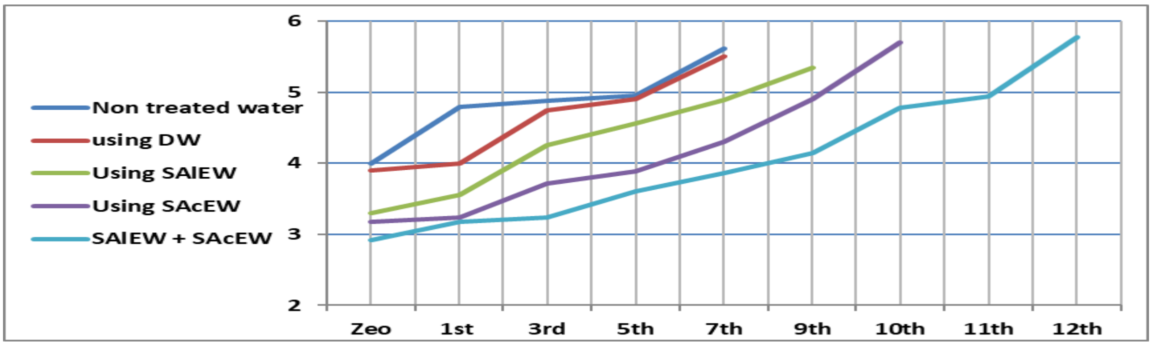

Fig 1. Effect of different water types on APC (Mean log_{10}cfu/g) and the shelf-life of chicken fillet

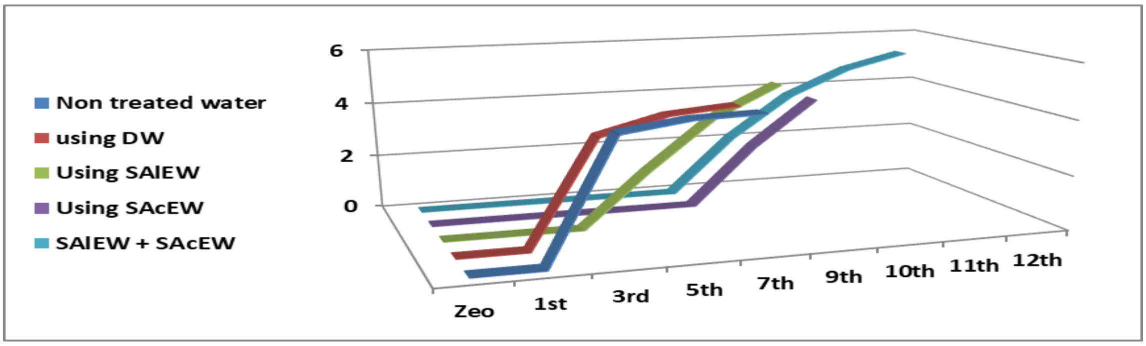

Fig 2. Effect of different water types on psychotropic count (Mean log_{10}cfu/g) and the shelf-life of chicken fillet

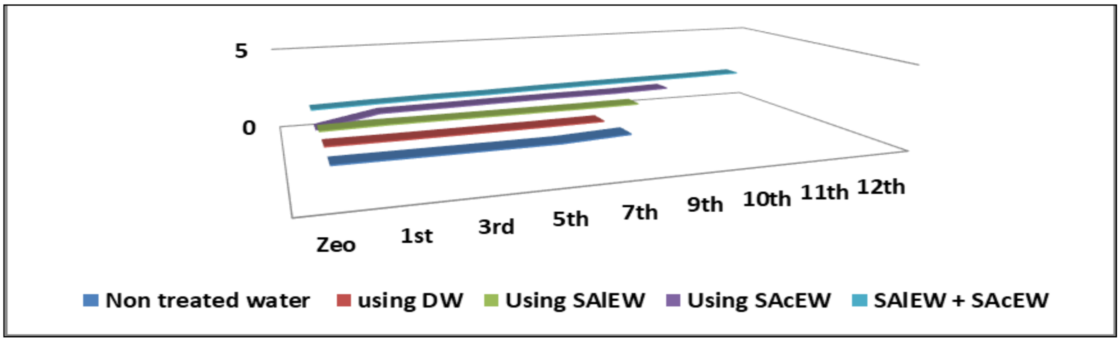

Fig 3. Effect of different water types on *E. coli* count (Mean log_{10}cfu/g) and the shelf-life of chicken fillet

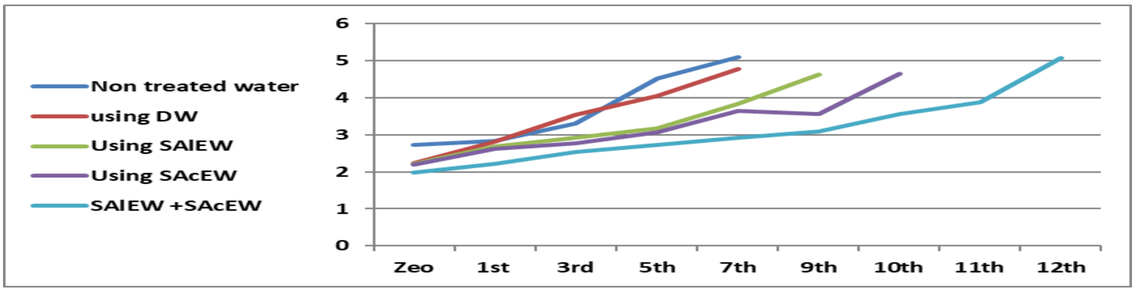

Fig 4. Effect of different water types on Mold count (Mean log_{10}cfu/g) and the shelf-life of chicken fillet

DISCUSSION

Results achieved in **Table (1) and Fig. (1)** revealed that APC in fresh chicken fillet without using of water dipping as well as that dipped in distilled water (DW) remained sound until the 5th day of storage as they recorded mean count log_{10} cfu/g±SD of 4.95±0.1 and 4.9±0.01, respectively, while the examined samples were unfit for human consumption at the 7th day of storage as they recorded 5.62±0.03 and 5.5±0.02. By using slightly alkaline and acidic electrolyzed water (SAlEW and SAcEW), the samples remained fit for consumption till 7th and 9th days (4.89±0.01 and 4.9±0.01), while they considered unfit for human consumption at the 9th and 10th days recording 5.35±0.06 and 5.70±0.01. Moreover, rinsing of chicken fillet by SAlEW followed by SAcEW resulted in prolong the shelf-life until the 11th day of storage (4.94±0.01 log_{10} cfu/g) and the samples were decomposed at the 12th day of storage (5.78±0.07 log_{10} cfu/g). These results agreed with **Vollmer et al. 2008 Koike et al. 2009 Jirotková et al. 2012 Rahman et al. 2012 Rasschaert et al. 2013 Rodrigo et al. 2015 Xiao-Ting et al. 2016 Duan et al. 2017 Xiaowei et al. 2018 Zang et al. 2019 Juan and José 2020 Attia et al. 2021 Federico et al. 2021 and Park et al. 2002**. who stated that SAcEW could effectively extend the shelf life of chicken meat and beef in comparison with that of other treatments. The SAcEW exhibited higher disinfectant and has bacterial inactivation efficacy compared with that of the distilled water treatment and control (without treatment), while **Fabrizio et al. 2002** and **Shimamura et al. 2016** hypothesized that combination treatment of SAlEW and SAcEW (4°C) reduced the microbial populations, which agreed with this study.

Also, **Table (1) and Fig. (2)** showed that Psychotropic count of non-water treated sample was <1 log_{10} cfu/g at zero time as well as the 1st day of storage, while the mean count recorded 4.30±0.04, 4.63±0.11 and 4.70±0.12 at the 3rd, 5th and the 7th day of storage, respectively. Meanwhile, the samples that rinsed with DW recorded <1 log_{10} cfu/g at zero time and the 1st day but the count increased from the 3rd day recording 3.80 ± 0.01, 4.40 ± 0.01 at the 5th day and 4.60 ± 0.02 at the 7th day of storage. In addition, the samples recorded <1 log_{10} at zero time, 1st and 3rd day when using SAlEW, while recorded 2.0±0.04, 3.8±0.02 and 4.9±0.02 for the 5th, 7th and 9th day of storage, respectively. Moreover, the results proved that rinsing with SAcEW remains the count <1 log_{10} till the 7th day and increased gradually recording 2.18±0.03 and 3.90±0.01 at the 9th and 10th day of storage, while by using SAlEW followed by SAcEW, the count recorded 2.11±0.01, 3.7±0.02, 4.67±0.02 and 5.23±0.01 at the 9th, 10th, 11th and 12 days, respectively. These results agreed with **Lee and Jang, 2004 and Attia et al. 2021** while disagreed with **Cichoski et al. (2019)** who stated that no significant differences between disinfection solutions and tap water treatment were detected for psychotropic bacteria.

The results in **Table (1) and Fig. (3)** confirmed that samples without water rinse recorded *E. coli* mean count (log_{10} cfu/g) of 1.95±0.01, 1.96±0.01, 1.97±0.01and 1.99±0.01 at zero time, 1st, 3rd, 5th days and spoiled at the 7th day of storage (2.16±0.01). While by using DWR, the count recorded 1.91±0.01, 1.92±0.02, 1.95±0.04, 1.98±0.01 and 2.03±0.05 for the aforesaid times, respectively. In this regard, SAlEW resulted in decline the count as matched with DWR to record 1.83±0.02, 1.91±0.02, 1.93±0.01, 1.95±0.01, 1.97±0.03) for the same aforementioned storage times in addition to 2.01±0.5 at the 9th day of storage at which the examined samples were spoiled. Moreover, SAcEW resulted in more declining of *E. coli* count as compared with SAlEW for the same periods (1.07±0.04, 1.77±0.02, 1.83±0.05, 1.90±0.01, 1.95±0.01, 1.98 ±0.02) and spoiled at the 10th day recording 2.08±0.04. While by using SAlEW followed by SAcEW, optimum results were recorded (1.48±0.03, 1.59±0.01, 1.67±0.03, 1.71±0.02, 1.80±0.02, 1.87±0.02, 1.93±0.03) in addition to 1.98±0.03 and 2.06±0.08 for the 11th and 12th day of storage, respectively. These results agreed with **Fabrizio et al. (2002)** and **Shimamura et al. (2016)** which reported that combination treatment of SAlEW and SAcEW at 4°C decreased the populations of *E. coli.*

In addition, the results in **Table (1) and**

Fig. (4) proved that samples without water rinse recorded mold count of 2.72±0.01, 2.83±0.06, 3.30±0.04 and 4.51±0.02 at zero time, 1st, 3rd, 5th days, respectively, and spoiled at the 7th day recording 5.1±0.03. Similarly, samples dipped in DW were spoiled at the 7th day recording 4.78±0.02. In this object, SAlEW kept the examined samples sound until the 7th day which recorded 3.84±0.02, but spoiled at the 9th day recording 4.62±0.01. By using SAcEW, mold count was declined in all storage days recording 2.19±0.04, 2.61±0.02, 2.78±0.01, 3.07±0.04, 3.64±0.04, 3.56±0.68 at zero time, 1st, 3rd, 5th, 7th and 9th day while spoiled at the 10th day recording 4.65±0.05. In addition, rinsing with SAlEW followed by SAcEW prolonged the shelf life of samples until the 11th day recording 3.88±0.03, while spoiled at the 12th day (5.08±0.59). These results agreed to those observed by **Jirotková et al. 2012 Xiong et al. 2014 and Lyu et al. 2018** as they concluded a significant decrease in the number of molds in chicken meat and wheat grains. Furthermore, the results found here differ from the data described by **Andrieli et al. 2020 and Lemos et al. 2020** who stated that EW cannot be considered effective in reducing molds count.

From the obtained results, it could be concluded that there were no significance difference (P>0.05) between samples without using water rinse and that rinsed with DW. On contrary, SAcEW showed a good reduction in microbial count more than SAlEW. This may be due to the fact that SAlEW acting as detergent while SAcEW act as sanitizer (**Deza et al. 2007 and Issa-Zacharia et al. 2009**. Moreover, the best results absolutely obtained ((P<0.05) by rinsing with SAlEW followed by SAcEW as compared with SAlEW or SAcEW separately.

In this respect, Japan's companies have developed products by electrolyzing a dilute hydrochloric acid solution in a diaphragm-less electrolytic cell and diluting with potable water to a pH of 5–6.5. This aqueous solution is used as a sanitizer. hypochlorous acid (HOCl) considered the major sanitizing component in slightly acidic electrolyzed water (SAcEW) which is differ from the hypochlorous acid solution produced by mixing an acid with sodium hypochlorite. In 2002, Japan's Ministry of Health, Labor, and in 2014, the Welfare designated SAcEW as a food additive sanitizer. Moreover, Ministry of Agriculture, Forestry, and Fisheries and the Ministry of the Environment authorized the use of SAcEW as control agent of microbial growth. Thus, areas which using SAcEW in Japan are in constant expansion, rapidly gaining acceptance and consumer satisfaction particularly in the area of food sanitation **(Kurahashi et al. 2021).** This came in accordance with the obtained results in the present investigation, which prove that using of SAcEW as a food sanitizer had good results in reduction of plenty of microorganisms.

Although, alkaline EW was effective in reducing the bacterial load of chicken fillet examined samples but its effect was not as strong as the sanitizing effect of acidic EW and therefore, SAlEW has its own applications for the industry and be strenthing when applied in sync before using of SAcEW.

Based on the results obtained in this research, the combination of SAlEW and SAcEW treatment is an effective and promising method for extending the shelf life of the chicken fillet meat when stored at 4±1°C, which agreed with **Fabrizio et al. 2002**; **Shigenobu and Seiichiro, 2007 and Shimamura et al. 2016** that evaluated the combination of AlEW and SAEW dipping treatment of chicken meat, and concluded significant reduction in bacterial counts. In this respect, **Huang et al. 2008** demonstrated that using electrolyzed water was successfully tested as disinfecting substance in the food industry.

CONCLUSION

There is investment in the food manufacturing system. Only tap water and kitchen salt (NaCl) are required to produce electrolyzed water solution. The main advantage over traditional sanitizing agents is that it is safe for humans and animals as well as it does not constitute even minor environment pollution. The system simply is a reactor (electrolyzer) technology that converts salt solution and water into sanitary solution through some reactions.

It is a potential alternative method of disinfecting, leading to lowering ammonia emissions into the stable environment as well as inhibit or suppress the growth of both spoilage and pathogenic microorganisms. Thus, using this innovative system allows for reduced usage of chemical compounds that may have adverse effect on human health and at the same time, it considered to be cost savings than other used disinfectants, less time consuming, easily prepared and be stable for long time when stored properly.

Electrolyzed Water can be effectively used to reduce microbial spoilage and therefore, extending the shelf life of chicken fillet meat and keep it in good quality condition during production, manufacturing distribution, storage and market handling. Also, EW can be implemented in the poultry processing plant as a green cleaner and disinfectant; it is appropriate system because it is efficient disinfection with no significant impact on the quality of the resulted raw materials in particular. Furthermore, because the pH of the waste fluid is neutralized (approximately pH 7.0) by continuously mixing SAlEW with SAcEW, the method has the advantage of not requiring any pH buffering adjustment; thus, the application of EW is considered environmentally friendly. Future researches should be focused on developing more effective dealing methods for controlling food poisoning bacteria in meat using electrolyzed water.

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