Malaysian Journal of Microbiology, Vol 18(3) 2022, pp. 282-290
DOI: http://dx.doi.org/10.21161/mjm.211343

Malaysian Journal of Microbiology
Published by Malaysian Society for Microbiology
(In SCOPUS since 2011)

Capability of acidic electrolyzed water in the elimination of *Salmonella* Typhimurium and *Escherichia coli* in the chicken breast

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Received 7 November 2021; Received in revised form 13 March 2022; Accepted 20 March 2022

ABSTRACT

Aims: This study aimed to investigate the effect of acidic electrolyzed water (AEW) as pre-refrigeration and pre-freezing processing steps for chicken meat in regard to the behavior of *S.* Typhimurium and *E. coli* during storage.
Methodology and results: AEW (free available chlorine 30 ppm and pH 2.7) was tested against *S*. Typhimurium and *E. coli* in growth media (brain heart infusion broth) and by exposing inoculated chicken fillets. The *in vitro* study appointed 10 minutes as the straightening exposure time of fresh prepared AEW for *S.* Typhimurium and *E. coli*. The reduction effect of AEW was significant (p<0.05) for both *S.* Typhimurium and *E. coli* along the 8 days of refrigerated storage with a maximum reduction after 24 h of post-treatment reaching 23.3% (1.4 log CFU/g) and 32.43% (2.15 log CFU/g) for *S.* Typhimurium and *E. coli*, respectively. AEW resulted in a significant reduction (p<0.05) as a pre-freezing application for both microorganisms, where the maximum reductions of 20% (1.2 log CFU/g) and 31.84% (2.14 log CFU/g) for *S.* Typhimurium and *E. coli*, respectively, were reported at zero time (just after dipping). In exposed samples to AEW, *S.* Typhimurium could not be detected by the 6th week of frozen storage while *E. coli* continued detectable until till 10th week but with a reduced population of 30% compared to control.
Conclusion, significance and impact of study: The findings of the present study suggest the application of AEW as a pre-refrigeration and pre-freezing treatment for chicken products. AEW application significantly improved the safety of chicken products.

Keywords: Electrolyzed, water, chicken, *Salmonella* Typhimurium, *E. coli*

INTRODUCTION

Food safety is a major issue for manufacturing of food to give healthful food to consumers as a kind of consumer protection. To achieve this goal, many of research works continue to find alternatives to traditional methods of controlling pathogens. One of these technologies is a green technology that aims to improve the physicochemical quality of food using natural resources with reducing chemical/toxic residues as possible (Proctor, 2011; Athayde *et al*., 2018). Electrolyzed water (EW) is a novel green technology that has arisen in recent years with potential applications in foods as an alternative to traditional pasteurization methods and heat treatments. It has the benefit of a wide range of food applications, including dipping and spraying (Athayde *et al*., 2018). Besides, it is safe for skin and mucous membranes, easy to handle (Al-Haq *et al*., 2005), generated quite quickly and easily (Jeong *et al*., 2007) in site, without environmental risk (Nakagawara *et al*., 1998; Tanaka *et al*., 1999) and of low production cost.

In Japan, EW has been included in the list of approved food additives since 2002 (Venturini, 2013). Furthermore, in the United States, the Environmental Protection Agency (EPA) has permitted the use of electrolyzed water in the food industry (Venturini, 2013). Besides, field studies have identified acidic electrolyzed water (AEW) as a promising prospective decontamination technique (Hao *et al*., 2012).

Poultry meat is a popular food source used all over the world and consumption has risen in many nations in recent decades. The comparatively economical cost of production, low-fat content and high nutritional value of poultry meat are some of the reasons for its value (Chouliara *et al*., 2007). Poultry now accounts for around 39% of global meat consumption, with only pork exceeding this share (FAO, 2022). Even though that chicken consumption is on the rise, the microbiological safety of chicken during storage and marketing is still a problem (Khalid *et al*., 2020). Consequently, different processing procedures have been employed to reduce bacterial contamination and increase the shelf life of

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ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 18(3) 2022, pp. 282-290
DOI: http://dx.doi.org/10.21161/mjm.211343

chicken during processing and storage in order to improve microbiological safety (Hwang and Beuchat, 1995; Göksoy *et al*., 2000; González-Fandos and Dominguez, 2007; Kim and Day, 2007).

Salmonella, *Listeria*, *Campylobacter* and *E. coli* are among the most common bacterial pollutants found in chickens' intestinal microflora (Anang *et al*., 2007). The existence of *Salmonella* was recorded in samples from chickens (Tarabees *et al*., 2017). Infection with *Salmonella* in commercial broilers in a surveillance study carried out from 2014 to 2015 was also reported (El-Sharkawy *et al*., 2017). Furthermore, the prevalence of *Salmonella* in chicken carcasses sampled from different locality markets was explored (Abdel-Aziz, 2016). *E. coli*, which is widely disseminated in intestinal environments (Joseph *et al*., 2002) is considered as the origin of serious clinical illnesses and mortality involved in outbreaks of foodborne disease (Bell, 2002). Besides, the genetic similarity of *E. coli* isolated from broilers with those associated with human infection was proved (Hussein *et al*., 2013) where the existence rate in samples of chicken viscera and human stool was reported to be 26.9% and 46.2%, respectively (Ramadan *et al*., 2016). Studies have also revealed a high occurrence of *E. coli* O157 in surveyed samples of meat products from different locations (Sallam *et al*., 2013; Ombarak *et al*., 2016).

This study aimed to determine an optimum dipping duration of AEW for *E. coli* and *S.* Typhimurium. Secondly, to evaluate the efficacy of AEW as a pre-refrigeration and freezing treatment to control *E. coli* and *S. Tyhimurium* loaded in chicken breasts via monitoring their ability to survive during refrigerated and frozen storage.

MATERIALS AND METHODS

Preparation of acidic electrolyzed water (AEW)

AEW (pH of 2.7 and free available chlorine ACC of 30 ppm) was produced by electrolysis of tap water brined with sodium chloride (3%). The electrolysis chamber with two poles, anode (aluminum) and cathode (carbon) were separated into two sides (Huang *et al*., 2008). The exchange of ions occurs between two separate sides through a bridge containing a saturated solution of sodium chloride, where electrodes provided with direct current voltage (9-10 V and 8-10 A) run for 10 min. At the anode side, the acidic electrolyzed water was formed which was used in the experiments. The pH level of formed acidic electrolyzed water was estimated using a digital pH meter (FSSAI, 2015). Also, ACC was estimated by chlorine test kit, according to Farah and Ali (2021).

Bacterial strains

Salmonella Typhimurium (NCTC12023) and *Escherichia coli* (ATCC25922) were obtained from Animal Health Research Institute, Dokki, Giza, Egypt. All of the strains were stored chilled on tryptic soy agar (TSA) slants and activated separately in 9 mL of tryptic soy broth (TSB) at 37 °C for 24 h prior to the experiment. Then strains were streaked on xylose lysine deoxycholate (XLD) agar for *S.* Typhimurium and eosin methylene blue (EMB) agar for *E. coli* and kept at 37 °C for 24 h. Colonies were confirmed by microscopic examination (Gram-stained smears) and biochemical reactions.

Preparation of bacterial inoculums

Two to three separate colonies of each *S*. Typhimurium and *E. coli* strains from overnight culture on their selective solid medium were transferred into separated 10.0 mL brain heart infusion (BHI) broth and incubated at 37 °C for 20 h. Each strain culture in BHI broth was diluted to approximately match 1.5×10^8 CFU/mL (0.5 Mcfarland) and further confirmed by counting on agar plates.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The MIC and MBC of AEW for *S.* Typhimurium and *E. coli* strains were determined using the broth dilution method (Quinn *et al*., 2004). Where a set of two-fold dilution tubes each contain 2 mL of two-fold serially diluted (100-1.62% v/v) AEW in Mueller Hinton broth. The tubes together with control tubes containing broth without AEW (positive control) and another containing 70% (v/v) ethyl alcohol (negative control) were inoculated by calculated bacterial inoculum of 10^7 CFU/mL of target bacteria (*S.* Typhimurium or *E. coli*). Post thorough mixing, the tubes were incubated at 37 °C for 24 h. The lowest concentration of AEW at which no growth (no turbidity in the tube) was observed and recorded as the MIC value. The MBC values were determined by subculturing of all tubes showed no visible signs of growth/turbidity into sterile Mueller Hinton agar plates and further overnight incubation at 37 °C. The MBC value of the tested AEW against the tested bacterial strain was the lowest concentration of AEW that resulted in no growth.

Time-kill assay

The time-kill assay was carried out in accordance with the Clinical and Laboratory Standards Institute (CLSI, 2018). In order to assess the time-kill effect of AEW against *S.* Typhimurium and *E. coli*, the particular bacterial suspension was mixed with sterile distilled water and standardized using McFarland 0.5 to obtain a colony-forming unit (CFU) of 10^7. The bacteria suspension (calculated inoculum 10^7 CFU/mL) was then added separately into a respective test tube containing freshly prepared AEW. Sterile distilled water with the same inoculum was used as control. At time interval started from zero time (just after inoculation) then every 2 min, 1 mL of the bacteria suspension was taken from particular test tubes and ten-fold dilution serially diluted using normal saline. Then 0.1 mL from each dilution plated onto Mueller Hinton agar plates followed by incubation at 37 °C for 24 h. All procedures were carried out three times. The time-kill analysis was used to measure the AEW

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 18(3) 2022, pp. 282-290
DOI: http://dx.doi.org/10.21161/mjm.211343

bactericidal impact by manually counting the bacteria colony growth in each agar corresponding the sampling duration of the experiment.

Studying the effect of AEW on pathogens loaded on chicken breast

Sample preparation and groups design

Fresh, raw chicken boneless breasts were obtained from a local slaughterhouse in Assiut, Egypt and kept refrigerated at 4 °C prior to being used for the experiment (within 3 h). Breast muscles were divided into 2 cm thickness fillets. Then fillets were sectioned into sample pieces each 3 cm × 3 cm (10 g each). The sample pieces are divided into 8 groups; half of them (4 groups) represent the part of the samples held at refrigerated temperature and the other half for a frozen part. Each half comprises two groups for inoculation of *S.* Typhimurium (first group as a control and second for AEW treatment) and similarly other two groups for inoculation of *E. coli* strains.

Samples inoculation and treatment application

Each of the chicken breast pieces was surface inoculated with the target bacterium (*S.* Typhimurium or *E. coli*) by calculated inoculums of 10^7 CFU/g of bacterial suspensions by dribbling on the flesh surface then spread by a sterile bent glass rod. Following inoculation, samples were allowed to absorb the inoculum inside a biosafety cabinet for 15 min. After that, as a groups design, intended groups for AEW treatment were dipped separately for 10 min in freshly prepared AEW at room temperature (22 ± 2 °C). While controls groups were dipped in sterile distilled water. Dipped samples were drained for 1 min then individually packaged in polyethylene terephthalate containers (Al-Holy and Rasco, 2015).

The two inoculated control groups (*S.* Typhimurium and *E. coli*) and similarly, two AEW treatment inoculated groups were stored at refrigerated temperature (4 °C). The other four groups were stored at frozen temperature (–18 °C). Assessment of treatments and control was carried through counting survivors of *S.* Typhimurium and *E. coli* immediately after AEW treatments (day 0) and at the storage intervals (1, 2, 4, 6, 8 and 10 days) for samples stored at refrigerated temperature (4 °C). While for samples stored in freezing (–18 °C) at storage intervals (1, 2, 4, 6, 8, 9 and 10 weeks).

Statistical analysis

In each test, the mean and standard deviation values were determined for each group. In order to compare two groups in unrelated samples, the Mann-Whitney method was utilized. The significance level was set at $p \leq 0.05$. Statistical analysis was performed with IBM® SPSS® Statistics Version 26 for Windows.

Table 1: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of acidic electrolyzed water against *S*. Typhimurium and *E. coli*.

Tests/stains	*S*.Typhimurium	*E.coli*
MIC	A. C.	A. C.
MBC	A. C.	A. C.

A. C.: Absolute concentration.

RESULTS AND DISCUSSION

Available chlorine concentration (ACC) of AEW is the main determinant of its efficacy. Free chlorine species (e.g., ClO, Cl_2, HClO) were reported as the potential antibacterial structures in EW (Huang *et al*., 2008). Meanwhile, AEW activity is attributed to HOCl, indirectly, as OH- is generated after HOCl permeation in the bacterial cells (Mokudai *et al*., 2012; Mokudai *et al*., 2015). HOCl is significant because the chlorine in the Cl_2 form may volatilize, as well as having an 80-fold higher sanitizing action than OCl (Eifert and Sanglay, 2002).

In the present study, *in vitro* investigation of antibacterial activities of AEW against *S.* Typhimurium and *E. coli* revealed that only AEW in undiluted condition (100%) appeared inhibitory and lethal effects (Table1). Related research studies have revealed EW to be an efficient sanitizer. It appeared as a bactericidal to *E. coli* O157:H7 (Park *et al*., 2004) and *Salmonella* Typhimurium (Fabrizio and Cutter 2003). The bactericidal effects of slightly AEW against *E. coli* occur through the cellular and biochemical mechanisms of cell necrosis and apoptosis (Ye *et al*., 2017). However, comparing results from different assays of EW in growth media are relatively difficult, due to the differences in methodology and adopted conditions. In addition, the sensitivities of food-related pathogens towards EW are varying (Rahman *et al*., 2016).

To specify the required dipping time for effective antibacterial application, the time-kill measurements of prepared AEW on *S.* Typhimurium and *E. coli* were carried out as complementary to the *in vivo* investigation. Previous studies suggest that besides chlorine concentrations, other factors, such as dipping time, must be adjusted (Arevalos-Sánchez *et al*., 2013; Al-Holy and Rasco, 2015).

When AEW contacted with *S.* Typhimurium at the initial time (0 min), a 1.12 log CFU/mL reduction compared to control was achieved (Figure 1). The reduction increased along the increasing time of exposure of *S.* Typhimurium to AEW. The recorded reductions were 1.68, 1.97, 2.78, 3.96 and 4.2 log CFU/mL at 2, 4, 6, 8, 10 min, respectively as recorded in the time-kill curve. The peak of the reduction curve was reached at 10 min exposure with a negligible variation (0.24 log CFU/mL) compared to reduction at 8 min. That indicated 10 min could be set as a proper exposure dipping time of AEW on *S.* Typhimurium in food application.

Concerning the time-kill curve of prepared AEW for *E. coli*, the data of Figure 2 cleared that with comparison to

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 18(3) 2022, pp. 282-290
DOI: http://dx.doi.org/10.21161/mjm.211343

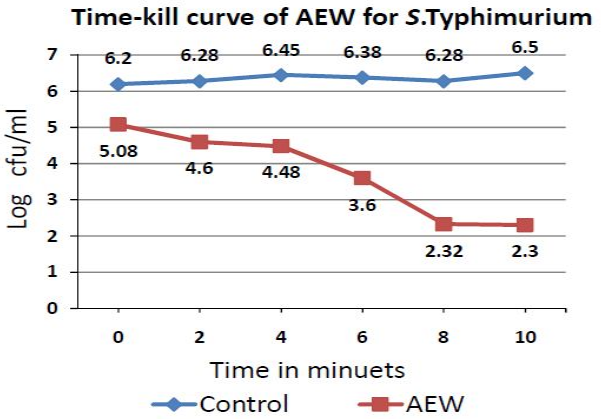

Figure 1: Time-kill curve of AEW for *S.* Typhimurium.

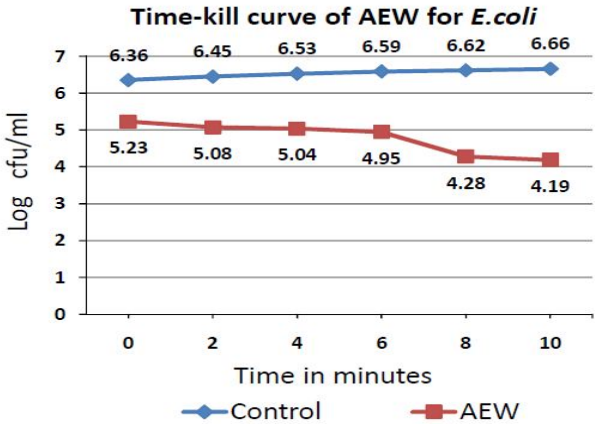

Figure 2: Time-kill curve of AEW for *E. coli*.

control. The reduction reached its peak after 10 min of exposure. Meanwhile, there is almost constancy in reduction at 8 and 10 min which was 2.34 and 2.47 log CFU/mL, respectively, with slight variation (0.13 log CFU/mL). This appointed 10 min as straighten dipping time required for *E. coli* exposure.

Salmonella in broiler meat is thought to constitute a severe health danger to the public (Evers, 2004). The percentage of carcasses positive for *Salmonella* varies significantly from nation to country, ranging from 5% to 21% (Kegode *et al*., 2008) considering the wide temperature range (5-47 °C) in which *Salmonella* may grow (Doyle, 1989). Also Wilks *et al*. (2005) reported that at low temperatures, *E. coli* can persist for a long duration (28-60 days). Meanwhile, Jackson *et al.* (2007) avowed that refrigerated storage requires antibacterial intervention.

AEW was applied in the current study as a decontaminant treatment against *S.* Typhimurium and *E. coli* loaded on chicken fillets stored at refrigerated temperature (4 °C). A significant reduction in their counts ($p \leq 0.05$) along most of 10 days of refrigerated storage could be obtained (Table 2 and Table 3). The maximum reductions were recorded after 24 h of post-treatment refrigeration which was 23.3% (1.4 log CFU/g) and 32.43% (2.15 log CFU/g) for *S*. Typhimurium and *E. coli*, respectively. With the slight increase from the initial reduction at zero time 20.16% (1.21 log CFU/g) and 31.84% (2.14 log CFU/g) for *S*. Typhimurium and *E. coli*, respectively. The initial count reduction of *S.* Typhimurium in the present assay was near to (1.9 log CFU/g) that recorded by Rahman *et al.* (2012) for inoculated chicken breast and (1.7 log CFU/g) of inoculated fresh pork recorded by Fabrizio and Cutter (2004). Regarding *E. coli*, the current results of initial count reduction around 1.7 log CFU/g that founded by Northcutt *et al*. (2007) for inoculated chicken carcasses after EW washing. In a related study, Al-Holy and Rasco (2015) reported that by exposing E. *coli* O157:H7 on chicken and beef samples for 5 or 10 min of AEW treatment, the reduction was proportional to concentration. Controversially, Shimamura *et al.* (2016) recorded that the *E. coli* reduction exceeded 3.0 log CFU/g at 4 °C for 3 min treatment of inoculated chicken breasts with AEW. The variation in reduction could be attributed to ACC. In the same respect, Park *et al.* (2004) demonstrated that higher ACC led to a higher reduction of *E. coli* by EW. Moreover, Phuvasate and Su (2010) reported that EW containing 100 ppm of chlorine was shown to be more effective in reducing bacteria than EW containing 50 ppm of chlorine. Furthermore, Park *et al*. (2002) related the antimicrobial properties of acidified EW to synergistic effects of the low pH and high chlorine content.

The reduction of *S.* Typhimurium (Table 2) showed fluctuation until the 4th day then illustrated by referring to partially constant of *S.* Typhimurium count in AEW samples in 4th, 6th and 8th days (3.8, 3.7 and 3.8 log CFU/g) compared to count increase of control sample and that demonstrated by significant difference between days in 2nd day until 8th days. The explanation came from Jiménez *et al.* (2009), who reported that by proceeding of storage at 8 °C, *Salmonella* inoculated on chicken skin multiplied slowly. Nearly the same observation was also reported by Rahman *et al.* (2012) where *S*. Typhimurium showed survival but could not grow by the interval of 10 days storage period.

By 10 days sampling interval of the current study, the reduction becomes insignificant and decreases to 10.17% (0.6 log CFU/g). That coincides with Rahman *et al*. (2012) findings (0.4 log CFU/g reduction) at the end of 10 days storage for meat samples treated with EW and stored at 5 °C. Meanwhile, Fabrizio *et al.* (2002) demonstrated that EW can reduce *S*. Typhimurium on poultry surfaces following extended refrigerated storage.

To some extent, the *E. coli* show the same manner of reduction as on the 2nd day, the reduction was 18.61% which was below that on the 1st day (Table 3) but still insignificant, later on became partially constant on the 4th, 6th and 8th days and that attributed to steady of *E. coli* count in AEW treated samples comparing to control count which slight decrease along the storage period. That is closely adjacent to the result reported by Arias *et al*. (2001) that within 72 h, *E. coli* counts dropped by almost a 1 log at 0 and 6 °C. By the end of the storage

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 18(3) 2022, pp. 282-290
DOI: http://dx.doi.org/10.21161/mjm.211343

Table 2: Effect of acidic electrolyzed water (AEW) on *S.* Typhimurium count in chicken fillets during refrigerated storage.

Sampling intervals	Statistical values of *S.* Typhimurium during refrigerated storage					
	Control Mean values (log CFU/g)	AEW Mean values (log CFU/g)	*p* value[1]	Reduction (log CFU/g)	Reduction %	*p* value[2]
Zero day	6.00	4.79	0.017*	1.21	20.16	
1st day	6.00	4.60	<0.001**	1.40	23.30	0.833
2nd day	4.97	4.20	0.002**	0.77	15.49	0.044*
4th day	4.84	3.84	<0.001**	1.00	20.66	0.019*
6th day	4.78	3.70	0.022**	1.08	22.50	0.014*
8th day	5.00	3.84	<0.001**	1.15	23.00	0.035*
10th day	5.90	5.30	0.159	0.60	10.17	0.588

*: Significant difference (p<0.05); **: Highly significant difference (p<0.01); *p* value[1]: Difference between control and AEW; p value[2]: Difference between days reduction.

Table 3: Effect of acidic electrolyzed water (AEW) on *E. coli* count in chicken fillets during refrigerated storage.

Sampling intervals	Statistical values of *E. coli* during refrigerated storage					
	Control Mean values (log CFU/g)	AEW Mean values (log CFU/g)	*p* value[1]	Reduction (log CFU/g)	Reduction %	*p* value[2]
Zero day	6.72	4.58	<0.001**	2.14	31.84	
1st day	6.63	4.48	<0.001**	2.15	32.43	0.892
2nd day	5.48	4.46	0.008**	1.00	18.61	0.237
4th day	5.18	4.20	0.009*	0.97	18.8	0.102
6th day	5.00	4.00	0.004**	1.00	20.00	0.057
8th day	4.88	3.95	<0.001**	0.93	18.06	0.040*
10th day	4.48	3.70	0.001**	0.78	17.41	0.012*

*: Significant difference (p<0.05); **: Highly significant difference (p<0.01); *p* value[1]: Difference between control and AEW; *p* value[2]: Difference between days reduction.

period (10 days) of the current study, the reduction of *E. coli* count was decreased (17.41%) with significant differences between days. The results of the present AEW assay against *E. coli* were close to those found by Jadeja *et al*. (2013) and Gómez-López *et al*. (2015) as they confirmed that AEW is efficient against *E. coli*.

Dave and Ghaly (2011) mentioned that bacterial viability in foods at low temperatures is also influenced by the nutritional makeup. For example, fresh chicken meat, fish meatballs, nuggets and peas have a high nutritional content, allowing practically all bacteria to flourish, resulting in increased competition. That makes a persistent demand for AEW application to control *S.* Typhimurium and *E. coli* as the most serious food poisoning microorganisms. Besides that, from the aspect of food safety, it's important to remember that a microbiological danger is determined not only by the prevalence but also by the concentration of the pathogen detected in a particular product (Jiménez *et al*., 2009).

Freezing of meat products may give additional protection against food pathogen infections by destroying a percentage of any potentially infective cells (Modi *et al*., 2006). Despite frozen food have a remarkably good safety record, related food poisoning with such food could happen (Archer, 2004). Freezing conserves the quality of foods, besides vitality of some pathogenic bacteria can be preserved (Archer, 2004). Psychrophilic and psychrotrophic bacteria are of great interest in food spoilage. They are microorganisms characterized by their complex metabolic pathways to adapt to extreme conditions of life (Vasut and Robeci, 2009). It dominates on the surface of meat stored at low temperatures. When it exceeds 10^8/cm^2 skin, it results in rejection of the product (Kraft, 1992). Consequently, there is a need for applying the antimicrobial approach to food that will be subjected to freezing.

In this work, the studying effect of pre-freezing treatment AEW on *S.* Typhimurium and *E. coli* showed that the maximum significant reduction between treatment (p≤0.01) of both pathogens occur on initial zero time at room temperature before undergoing freezing which was 20% (1.2 log CFU/g) and 31.84% (2.14 log CFU/g) respectively (Table 4 and Table 5).

Concerning *S.* Typhimurium (Table 4), after a one-week interval of freezing, the reduction compared to control decline from that recorded at zero time but still significant (14.57%) and continued significant reduction (p≤0.05) on 2nd week (13.88%) before becoming insignificant between treatments on 4th week (1.18%). In the time, there is a significant difference between week's intervals. The population *S*. Typhimurium appeared steady count in treated AEW samples at 1st, 2nd and 4th weeks which be recorded as 4.34, 4.28, 4.2 log CFU/g, respectively. From the 6th week until the end of the storage period at the 10th week, *S*. Typhimurium count was recorded as undetectable in both AEW treated and

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 18(3) 2022, pp. 282-290
DOI: http://dx.doi.org/10.21161/mjm.211343

Table 4: Effect of acidic electrolyzed water (AEW) on *S.* Typhimurium count (log CFU/g) in chicken fillets during frozen storage.

Sampling intervals	Statistical values of *S.* Typhimurium during frozen storage					
	Control Mean values (log CFU/g)	AEW Mean value (log CFU/g)	*p* value[1]	Reduction (log CFU/g)	Reduction %	*p* value[2]
Zero Time	6.00	4.80	0.001**	1.20	20.00	
1st week	5.08	4.34	0.014*	0.74	14.57	0.063
2nd week	4.97	4.28	<0.001**	0.69	13.88	0.034*
4th week	4.25	4.20	0.288	0.05	1.18	0.002**
6th week	N.D.	N.D.	-	-	-	
8th week	N.D.	N.D.	-	-	-	
9th week	N.D.	N.D.	-	-	-	
10th week	N.D.	N.D.	-	-	-	

N.D: Not detected; *: Significant difference (p<0.05); **: Highly significant difference (p<0.01); *p* value[1]: Difference between control and AEW; *p* value[2]: Difference between weeks reduction.

Table 5: Effect of acidic electrolyzed water (AEW) on *E. coli* count (log CFU/g) in chicken fillets during frozen storage.

Sampling intervals	Statistical values of *E. coli* during frozen storage					
	Control Mean values (log CFU/g)	AEW Mean values (log CFU/g)	*p* value[1]	Reduction (log CFU/g)	Reduction %	*p* value[2]
Zero Time	6.72	4.58	<0.001**	2.14	31.84	
1st week	4.30	4.00	0.176	0.30	6.98	0.012*
2nd week	4.30	3.48	<0.001**	0.82	19.07	0.007**
4th week	4.30	3.48	0.002**	0.82	19.07	0.007**
6th week	4.11	3.48	<0.001**	0.63	15.33	0.004**
8th week	3.11	2.78	0.025*	0.33	10.61	<0.001**
9th week	3.00	2.30	0.018*	0.70	23.3	<0.001**
10th week	3.00	2.10	<0.001**	0.90	30.00	<0.001**

*: Significant difference (p<0.05); **: Highly significant difference (p<0.01); *p* value[1]: Difference between control and AEW; *p* value[2]: Difference between weeks reduction.

control samples. By exposure to stresses of freezing, microorganisms can suffer reversible or irreversible mechanical damages. Injury of cells can be produced by either metabolic damage (Ray and Speck, 1973) or by the intracellular formation of ice crystals through rapid freezing of cells (El-Kest and Marth, 1992). In the same respect, Dominguez and Schaffner (2009) recorded that frozen storage of chicken products for 16 weeks; was a possible mean of damage to *Salmonella*.

Regarding *E. coli* (Table 5), with the exception of on 1st week sampling interval, the reduction in their population continued significantly between treatments (p≤0.05) along the 10th week of the freezing period. Also Arias *et al*. (2001) and Restaino *et al*. (2001) recorded that with the most rapid cellular death and injury of *E. coli* populations, injury in beef infusion storage at –25 °C was reported in the first 10 days of storage. The *E. coli* count of AEW treated sample recorded the same count (3.48 log CFU/g) on 2nd, 4th and 6th week with raise reduction 19.07%, 19.07% and 15.33%, respectively, with a significant difference between week's intervals. At the end of the freezing storage period, the count of control and AEW treated samples was 3 and 2.1 log CFU/g, respectively with a reduction of 30% (0.9 log CFU/g). Manios and Skandamis (2015) concluded that frozen storage enhanced the survival of *E. coli*, as they recoded 3.1 log CFU/g on 75 days. That pronounced the demand of AEW in *E. coli* control in frozen storage. The current reported results regarding the efficiency of AEW to reduce *S.* Typhimurium and *E. coli* in the frozen chicken breast coordinates with findings of Loi-Braden *et al*. (2005) that pre-freezing treatment of shrimp by AEW followed by freezing at –20 °C resulted in a significant reduction in *E. coli* and *Salmonella* populations.

CONCLUSION

The findings of the present study suggest the application of AEW as a pre-refrigeration and pre-freezing treatment for chicken products. The green intervention of the AEW application significantly improved the safety of chicken products due to achievable antibacterial efficiency against *S.* Typhimurium and *E. coli*. As food safety regulation of European Communities, No 2073/2005 ensures the effective measures are taken to control and reduce *Salmonella* and *E. coli* at relevant stages of the food chain**.** Further study should be conducted to investigate the antibacterial efficiency of AEW on most psychrophilic and psychrotrophic food poisoning bacteria.

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

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 ISSN (print): 1823-8262, ISSN (online): 2231-7538