Inactivation of *Salmonella* Typhimurium on poultry meat by electrolyzed water

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Abstract: *Salmonella* is the most frequent causes of food poisoning in humans. Eggs and poultry are the main common sources of such outbreaks. One of the most commonly isolated sero-types from poultry is *S.* Typhimurium. A number of interventions are used extensively by the meat and poultry industries to reduce bacterial contamination. Organic acids, chlorinated compounds, trisodium phosphate, heat, steam or hot water are generally recognized as safe (GRAS) interventions and are used extensively by the meat and poultry industries to reduce bacterial contamination on carcass surfaces. Electrolyzed Water (EW) is currently gaining popularity as a sanitizer in the food industry. Generation of EW, in general, involves reactions in a cell containing inert positively charged (anode) and negatively charged (cathode) electrodes, respectively, separated by a membrane, and through which a dilute salt solution passes. The objective of this study was to determine the efficacy of EW in the inactivation of *Salmonella* Typhimurium in chicken meat and monitor the effects during the shelf –life. Chicken wings were inoculated with two types of *Salmonella* Typhimurium, ATCC 14028 and a wild strain. Inoculated samples dipped in three different EW that include 30, 60 and 70 ppm chlorine, for 15, 30 and 60 seconds. Chicken wings were sampled at day 0 in order to determine the antimicrobial effect, the rest of the samples stored in 7 °C for 3 and 7 days to monitor the effect of shelf-life. As a result, EW reduced the *Salmonella* Typhimurium approximately 2 log CFU/ml on day 0. However, no reduction observed during the 7 days. There was no difference between 30, 60 and 70 ppm chlorine included EW's, and also 15, 30 and 60 seconds.

Key words: Decontamination, electrolyzed water, *Salmonella* Typhimurium

Elektrolize su kullanılarak tavuk etinde *S.* Typhimurium'un inaktivasyonu

Öz: Gıda kaynaklı infeksiyonlara neden olan etkenlerin başında *Salmonella* yer almaktadır. *Salmonella*'nın en önemli bulaşma kaynağı ise kanatlı etleri ve yumurtadır. Kanatlı etleri arasında sıklıkla izole ve identifiye edilen serotiplerden birisi de *S.* Typhimurium'dur. Et ve et ürünleri sanayiinde, dekontaminasyon uygulamalarında organik asitler, klorlu bileşikler, trisodyum fosfat, buhar veya sıcak su GRAS Kabul edilen ve mikroorganizmalara karşı en sık kullanılan maddelerdir. Elektrolize su da gıda endüstrisinde kullanımı yaygınlaşmaya başlamış yeni bir sanitizerdir. Elektrolize su, seyreltik tuz çözeltisinin membranla ayrılmış anot ve katot elektrotlarından geçirilerek elektrolizi ile elde edilmektedir. Bu çalışma, elektrolize suyun tavuk etlerinde *S.* Typhimurium üzerine etkisini ve raf ömrü boyunca antimikrobiyel etkinin ne şekilde geliştiğini gözlemlemek amacıyla yapılmıştır. Tavuk eti örnekleri ATCC 14028 ve saha izolatı ile kontamine edilmiştir. Kontamine edilen numunelere, 3 farklı grup şeklinde 500'er ml 30, 60 ve 70 ppm klor içeren solusyonlar ile

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dekontaminasyon işlemi uygulanmıştır. Her gruptaki numuneler 15, 30 ve 60 saniye olmak üzere 3 farklı zaman parametresinde dezenfektanlarda bekletilmiştir. Süreler sonunda numunelerin 0. ve +7 °C de bekletildikten sonra 3. ve 7. günlerde ekimleri yapılarak kontaminasyon düzeyleri tespit edilmiştir. Sonuç olarak elektrolize suyun 0. günde 2 log bir düşüş sağlarken, 3. ve 7. günlerde etkisini kaybettiği ortaya konmuştur. Ayrıca çalışmada kullanılan değişik elektrolize su konsantrasyonları ve uygulama süreleri arasında da bir fark olmadığı tespit edilmiştir.

Anahtar Sözcükler: Dekontaminasyon, elektrolize su, *S.* Typhimurium

Introduction

Salmonella infection is a major cause of gastroenteritis in humans (salmonellosis) worldwide and is often associated with consumption of raw or undercooked poultry meat (13). A large number of *Salmonella* serotypes have been associated with poultry meat and the top 4 serotypes are Enteritidis, Typhimurium, Newport and Javiana (1). Extensive experience, research and field trials have identified a diversity of management and intervention strategies for the reduction and, potentially, elimination of enteropathogens, such as *Salmonella* from poultry (2). Organic acids, chlorinated compounds, trisodium phosphate, heat, steam or hot water are generally recognized as safe (GRAS) interventions and are used extensively by the meat and poultry industries to reduce bacterial contamination on carcass surfaces (3). EW is currently gaining popularity as a sanitizer in the food industry on foods and processing surfaces (9). EW water was initially developed in Japan and it has been reported to have strong bactericidal effects on most pathogenic bacteria that are important to food safety (5). Generation of EW, in general, involves reactions in a cell containing inert positively charged (anode) and negatively charged (cathode) electrodes, respectively, separated by a membrane, and through which a dilute salt solution passes. By subjecting the electrodes to direct current voltage, negatively charged ions such as hydroxide and chloride in the salt solution move to the anode to give up electrons and become oxygen gas, chlorine gas, hypochlorite ion, hypochlorous acid and hydrochloric acid, while positively charged ions such as hydrogen and sodium move to the cathode to take up electrons and become hydrogen gas and sodium hydroxide. As a result, two types of water possessing different characteristics are generated. An electrolyzed basic solution [pH>11 and oxidation–reduction potential (ORP) < -800 mV] is produced from the cathode side, which has strong reducing potential and may be used as a cleaning solution. An electrolyzed acid solution (pH<2.7 and ORP>1100 mV and presence of hypochlorous acid) is produced from the anode side, which has strong oxidation potential and bactericidal effect and can be used as a disinfectant (4). The major advantages of using EW are less adverse environmental impacts and without the difficulties of transporting and storing potentially hazardous chemicals. It is safe for staff, non-irritating, has minimal toxicity and low cost (8). The only disadvantages are nonstable and its bacterial effect decreased in the presence of organic matter (12).

United States Department of Agriculture (USDA) approved the use of EW in meat and poultry products and eggs. Also United States Environmental Protection Agency (EPA) allowed the use of EW in raw foods. Food and Drug Administration accept EW as a disinfectant and declare that not against the usage in foods (3).

This study was designed to evaluate the effectiveness of EW for inhibiting *S.* Typhimurium and its potential application in reducing *S.* Typhimurium on chicken wings. The other objective of this study was to determine the efficacy of EW during the shelf –life.

Materials and Methods

Bacterial cultures

Salmonella Typhimurium ATCC 14028 and a wild type *Salmonella* Typhimurium were used for this study (obtained from Veterinary Control Central Research Institute Etlik – Ankara). Each strain was grown on Brillant-green phenol-red lactose sucrose (BPLS, Merck VM331547 140) agar at 37 °C for 24 h under an aerobic condition for counting the microbial load. After counting the microbial load, cultures were incubated at 37 °C for 24 hours in Brian Heart Infusion Broth (OXOID, CM0225). Then, each culture was diluted in sterile peptone water (OXOID, CM009) to obtain inoculums containing 10^5 cfu/ml bacteria.

Electrolyzed water

Commercial products which have different chlorine concentration, pH and ORP were used in the study. The chlorine concentration, pH and ORP of electrolyzed waters were, 30 ppm, 5.0, 925 mV; 50 ppm, 2.6, 1076 mV; 70 ppm, 2.2, 1100 mV, respectively.

Samples and inoculation

60 chicken wings were obtained from a local slaughterhouse and transported to the laboratory inside the coolers. For all experiments chinken wings surfaces were treated with UV light, surfaces were exposed evenly by turning every 10 min for up to 30 min. 0.1 ml of each culture, containing approximately 5 $\log_{10}$ CFU/ml, was inoculated onto UV-treated chicken wings external surfaces under a biological safety hood. Bacterial cultures were allowed to attached to chicken wings surfaces for 20 min at room temperature, prior to any treatments. Using this procedure approximately 3 $\log_{10}$ CFU/ml of *S.* Typhimurium ATCC 14028 and 4 $\log_{10}$ CFU/ml of wild type *S.* Typhimurium were obtained on chicken wings surfaces, respectively.

Treatment and bacteriological analysis of chicken samples

Inoculated chicken wings were placed individually in sterile bags containing 500 ml of three different electrolyzed water and shaken gently at room temperature for 15, 30 and 60 seconds. At the end of the treatment, each sample was placed immediately into 100 ml of sterile buffered peptone water (MERCK, VM323428 134) and rubbed gently with hands from the outside of the sterile bag for 2 min. Buffered peptone water were assayed through serially diluting in 9 ml of sterile peptone water and then directly plating 0.05 ml of each dilution in duplicate on BPLS and Xylose Lysine Desoxycholate Medium (XLD, OXOID, CM0469) and incubated at 37 °C for 24 h before counting (11).

Shelf-life study

Experimentally inoculated chicken wings were dipped in 500 ml of three different electrolyzed water at room temperature for 15, 30 and 60 seconds and stored at 7 °C in sterile bags and sampled at days 3 and 7. Sampling and microbiological analyses were performed as described above.

Statistical analysis

Data were analyzed by the General Linear Model procedure of the SPSS program for determine the effect of different chlorine concentration, time and days. Days were compared by ANOVA using Duncan's multiple range test to determine the significant differences.

Results and Discussion

As a result of this study, Table 1 and Table 2 shows the logarithmic microbial counts for *S.* Typhimurium ATCC 14028 and wild type *S.* Typhimurium, respectively. When numbers of bacteria recovered from control and EW dipped chicken wings were compared, only dip-

ping with 70 ppm EW for 60 seconds reduced levels of *S.* Typhimurium ATCC 14028 1 $\log_{10}$ CFU/ml. However, for wild type *S.* Typhimurium, except dipping with 30 ppm EW for 15 seconds, in all treatments reduction level was found 2 $\log_{10}$ CFU/ml. Statistical analysis showed that there was no significant difference between chlorine concentration and time for all treatments ($P > 0.05$). This study also investigated the efficacy of EW during the shelf –life. The results showed that after the day 0 all of the EW's lost their efficacy (Table 1 and Table 2).

Table 1. The logarithmic microbial counts (log 10) for *S.* Typhimurium ATCC 14028 on day 0, 3 and 7.

Tablo 1: *S. Typhimurium ATCC 14028 için 0., 3. ve 7. gün sonuçları.*

Days	Treatment	Time		
		15 sec	30 sec	60 sec
Day 0[A]	EW 1	3	3	3
	EW 2	3	3	3
	EW 3	3	3	2
Day 3[B]	EW 1	4	4	4
	EW 2	4	4	3
	EW 3	4	4	5
Day 7[C]	EW 1	6	6	6
	EW 2	5	6	5
	EW 3	5	5	5

EW 1: 30 ppm chlorine concentration, pH 5.0, ORP 925 mV; EW 2: 50 ppm chlorine concentration, p H 2.6, ORP 1076 mV; EW 3: 70 ppm chlorine concentration, pH 2.2, ORP 1100 mV. A: Microbial count for S. Typhimurium ATCC 14028 in control group is 3 $\log_{10}$ CFU/ml, B: Microbial count for S. Typhimurium ATCC 14028 in control group is 4 $\log_{10}$ CFU/ml, C: Microbial count for S. Typhimurium ATCC 14028 in control group is 6 $\log_{10}$ CFU/ml.

Table 2. The logarithmic microbial counts (log 10)for wild type *S.* Typhimurium on day 0, 3 and 7.

Tablo 2:*Wild tip S. Typhimurium için 0., 3. ve 7. gün sonuçları.*

Days	Treatment	Time		
		15 sec	30 sec	60 sec
Day 0[A]	EW 1	3	2	2
	EW 2	2	2	2
	EW 3	2	2	2
Day 3[B]	EW 1	4	4	4
	EW 2	4	4	4
	EW 3	4	4	4
Day 7[C]	EW 1	6	6	6
	EW 2	5	5	5
	EW 3	5	5	5

EW 1: 30 ppm chlorine concentration, pH 5.0, ORP 925 mV; EW 2: 50 ppm chlorine concentration, p H 2.6, ORP 1076 mV; EW 3: 70 ppm chlorine concentration, pH 2.2, ORP 1100 mV.**A:** Microbial count for wild type *S.* Typhimurium in control group is 4 $\log_{10}$ CFU/ml, **B:** Microbial count for wild type *S.* Typhimurium in control group is 4 $\log_{10}$ CFU/ml, **C:** Microbial count for wild type *S.* Typhimurium in control group is 6 $\log_{10}$ CFU/ml.

Different antimicrobial treatments are currently being used in meat industry to control and reduce pathogens. In this study the effectiveness of EW on *S.* Typhimurium was investigated and the changes during shelf-life was monitored. Data collected during the present study demonstrate that on the day 0, dipping chicken wings with EW is effective for reducing the number of bacteria. Moreover, the results show that, EW is not stable and losing its efficacy on day 3 and 7.

Several researchers investigated the effects of EW for inactivating and killing *E. coli* O157:H7*, Salmonella* spp., *C. jejuni* and *L. monocytogenes* on different kind of food. Fabrizio et al (2002), compare the effectiveness of EW with acetic acid and trisodium phosphate on poultry carcasses and reported that EW performed better than acetic acid and trisodium phosphate by immersion with nearly 4 $\log_{10}$ reduction of *S.* Typhimurium. In the study of Northcutt et al. (2007), spraying of poultry carcasses with EW yielded 2.7 $\log_{10}$ reduction on *Salmonella*. Kim et al (2005) investigated the efficacy of EW in preventing and removing fecal contaminants on poultry carcasses under simulated industrial processing conditions. Their results showed that prespraying chicken carcasses with EW significantly lowered cecal material attachment than tap water and 10% trisodium phosphate. Kumar et al (1999) detected the efficacy of EW in in vitro conditions. A five strain mixture of *S.* enteritidis of approximately 10^8 CFU/ml was inoculated in 9 ml EW and incubated at 4 and 23°C for 0, 5, 10 and 15 min. At 4 and 23°C, an exposure time of 5 min reduced the population by nearly 7 $\log_{10}$ and complete inactivation was detected at 10 min exposure.

Electrolyzed water shows its effect on pathogens due to high oxidation reduction potential, pH, and several forms of chlorine compounds. But these properties also make EW non-stable and susceptible against organic materials. Our findings supported that EW is not stable and losing its efficacy on day 3 and 7.

Electrolyzed water is a relatively new antimicrobial agent that has been shown to be effective against pathogen agents. The information from this study may provide poultry processors with an additional antimicrobial intervention to reduce pathogens on poultry carcasses. However, for practical application by the food industry, the efficacy of EW, needs to be further investigated.

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