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Efficacy of slightly acidic electrolysed water, chlorine dioxide, calcium oxide, and nisin against *Salmonella* Typhimurium and *Listeria monocytogenes* on chicken meats

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Abstract

The aim of the present work was to investigate the antibacterial effects of four different decontaminants [slightly acidic electrolysed water (SAEW), chlorine dioxide, calcium oxide, and nisin (N)] and two different application methods (spraying, S; and immersion, I) on *Salmonella* Typhimurium and *Listeria monocytogenes* strains on chicken meats. The decontamination effect of each solution was applied by spraying and immersion on days 0 (2 h after application), 1, 3, and 6. It was determined that SAEWI's antibacterial activity against *S.* Typhimurium continued from day 0 (0.85 log reduction) during the cold storage. It was observed that NS and NI applications decreased the number of *L. monocytogenes* by 2.82 - 2.76 log/g, respectively, on day 0 as compared to the control group; and had the strongest antibacterial effect in terms of sustaining the effect during cold storage. It was found that SAEWS had a weak antibacterial effect against *L. monocytogenes*, but had a stronger antibacterial activity against S. Typhimurium, the most important pathogen of poultry slaughterhouses and poultry meats. In conclusion, SAEWS is a decontaminant that could be included in *Salmonella* control programs in slaughterhouses, due to its antibacterial effect against *Salmonella* species.

Introduction

Approximately 47% of the death cases caused by foodborne bacteria are caused by non-typhoidal *Salmonella* and *Listeria monocytogenes* (Scallan *et al.,* 2011). In 2016, 839 foodborne disease outbreaks were reported, resulting in 14,259 illnesses, 875 hospitalisations, and 17 deaths in USA. *Salmonella* was the second most common cause, accounting for 132 (33%) outbreaks, and 3,047 (33%) illnesses (CDC, 2018). In the European Union countries, 94,625 individuals are reported to have been infected with salmonellosis, and 2,200 individuals are affected by listeriosis, of which 270 die every year (EFSA, 2016). Chicken meat is an ideal environment for the microorganisms to develop due to the nutritional substances it contains (water, proteins, lipids, vitamins, and minerals). During various stages of its production, chicken meat can be contaminated with various microorganisms, including *Salmonella* spp. and *L. monocytogenes* (Mead, 2004). Chicken carcasses are often contaminated with these pathogens due to the contact with faecal matter smeared over claws or feathers of the chicken, or inside of the intestines (Dinçer and Baysal, 2004). *Salmonella* spp. are persistent pathogens due to their ability to survive and multiply in a variety of environmental conditions. They can often survive disinfectant applications, resist desiccation, and develop biofilm to survive various *in vivo* and *in vitro* conditions (Vestby *et al.,* 2009; Mezal *et al.,* 2013). *L. monocytogenes* on the other hand, is reported to be capable of surviving for more than 12 years under non-optima environmental conditions (environmental temperature, a_w, pH, etc.), particularly in food production facilities (Holah *et al.,* 2004; Lambertz *et al.,* 2012).

It is therefore very important to reduce or prevent contamination of foodborne pathogens (particularly of *Salmonella* spp. and *L. monocytogenes*) in chicken meat production processes (Scallan *et al.,* 2011). Various decontaminants are used to prevent bacterial contamination in slaughterhouses. It is important that this decontamination procedure does not interfere with human health and alter the taste, aroma, and texture of the meats (Özbay and Sarıçoban, 2015). To this end, electrolysed water (Fabrizio *et al.,* 2002; Hricova *et al.,* 2008), chlorine dioxide (Novak

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et al., 2008), calcium oxide (Bae *et al.,* 2006), and nisin (Khalafalla *et al.,* 2016) are being utilised in decontamination process of various foods. Electrolysed water is amongst the highly preferred decontaminants for its ease of use, economical price, environment-friendly nature, and its strong bactericidal effects (Kim *et al.,* 2000; Park *et al.,* 2002; Huang *et al.,* 2008). The use of chlorine dioxide on food surfaces as a decontaminant was allowed by United States Food and Drug Administration (USFDA) due to its antimicrobial properties provided by the oxidising and disinfectant effects of its gas and liquid forms (Kochevar *et al.,* 1997, Annous *et al.,* 2001). Calcium oxide, the main component of calcium carbonate, is a substance with antibacterial properties that is obtained by heating scallop shells (Bae *et al.,* 2006). Nisin, on the other hand, is a bacteriocin produced by lactic acid bacteria, and can be used as bio-preservative in various foods (FDA, 2008; Khalafalla *et al.,* 2016).

The aim of the present work was to investigate the antibacterial effects of four different decontaminants (mild acid electrolysed water, chlorine dioxide, calcium oxide, and nisin) and two different application methods (spraying and immersion) on chicken breast meats artificially inoculated with *S.* Typhimurium and *L. monocytogenes*.

Materials and methods

Chicken breast meat samples

The chicken breast meat samples used in the present work were purchased from various markets in the province of Balikesir, Turkey. The samples were transported to the laboratory in cold chain (4°C) before analyses.

Reference strains

Salmonella Typhimurium ATCC 13311 was obtained from the culture collection of the Department of Food Hygiene and Technology, Faculty of Veterinary Sciences, Afyon Kocatepe University, Turkey. *Listeria monocytogenes* ATCC 7644 was obtained from the culture collection of the Faculty of Veterinary Medicine, Balikesir University, Turkey.

Artificial inoculation of chicken breast meat samples with S. Typhimurium and L. monocytogenes

Prior to inoculation, 25 g chicken breast meat samples were analysed for *Salmonella* spp. and *L. monocytogenes* presence. Samples that were negative for both pathogens were then inoculated with reference strains of *S.* Typhimurium and *L. monocytogenes*, which were incubated for 24 h at 37°C in Brain Heart Infusion Broth (Oxoid, UK). For artificial inoculation, each culture was diluted in sterile peptone water (Oxoid, UK) to obtain inoculums containing 10^7 CFU/mL bacteria. Next, 1 mL of each culture was inoculated onto 10 g chicken breast samples. Bacterial cultures were allowed to attach to chicken breast meats surfaces during cold storage (4°C) for 2 h. Using this procedure, approximately 10^6 CFU/g of S. Typhimurium and 10^5 CFU/g of *L. monocytogenes* were obtained on chicken breast meats, respectively.

Preparation of decontaminant solutions

Four different decontaminant solutions were tested in the present work; electrolysed water (10%, Proxityl), chlorine dioxide (0.3%, Cleanday), calcium oxide (10%, Calceramic®), and nisin (5%, Pronisin®). The decontaminant solutions were prepared using sterilised distilled water at room temperature. A digital pH meter (Hanna Instruments, USA) was used to determine the pH values of the decontaminant solutions. The pH values for the prepared decontaminant solutions are given in Table 1.

Table 1. The pH values of decontaminant solutions.

Solution name	pH
Slightly acidic electrolysed water (SAEW)	5.66 ± 0.09
Chlorine dioxide (ClO_2)	8.43 ± 0.35
Calcium oxide (CaO)	12.13 ± 0.20
Nisin	3.36 ± 0.05

Application of decontaminant to the contaminated chicken breast meat samples

The chicken meat samples were separated into nine groups for each bacteria. Each decontaminant solution was applied with two different methods of spraying (S) and immersion (I). The nine treatments were 1 = control (only inoculated with bacteria without decontaminant solution), 2 = sprayed with and 3 = immersed into slightly acidic electrolysed water (SAEWS and SAEWI, respectively), 4 = sprayed with and 5 = immersed into chlorine dioxide (ClO_2S and ClO_2I, respectively), 6 = sprayed with and 7 = immersed into calcium oxide (CaOS and CaOI, respectively), 8 = sprayed with and 9 = immersed into nisin (NS and NI, respectively). Spraying was performed in a manner that ensured all surface area of the chicken meat samples were sprayed for 20 s/100 mL, while immersion was performed by immersing the sample into the decontaminant for 10 min.

Microbiological analyses

Microbiological analyses of the chicken breast meat samples were performed before

inoculation, and at 2 h (0 d), 1, 3, and 6 d after inoculation.

Determination of pathogens before inoculation

The standard method ISO 6579-1 (ISO, 2017a) was used to isolate and identify *Salmonella* spp. Suspected *Salmonella* colonies were evaluated with Gram-staining and oxidase test. Gram-negative and oxidase negative isolates were inoculated onto Nutrient Agar (Difco, 0001-17), and incubated for 24 h at 30°C. Developed colonies were identified using API 20 E test kit (BioMérieux, France).

Isolation and identification of *L. monocytogenes* were performed following the ISO 11290-2 (ISO, 2017b) standard method. Three of the *Listeria*-suspected colonies were inoculated onto Nutrient Agar, and incubated for 24 h at 30°C. Developed colonies were identified using Oxoid™ Microbact™ Listeria 12L test kit (Thermo Fisher, USA).

Pathogen count after inoculation

Ten grams of chicken breast meat samples were taken into sterilised stomacher bags, and then was added with 90 mL Maximum Recovery Diluent (Merck, Germany) dilution liquid. These were then homogenised for 2 min in a stomacher (IUL Instruments, Spain). A series of dilutions were prepared from 10^{-1} to 10^{-5}. From each dilution factor, inoculations were performed onto XLD agar (for *S.* Typhimurium), and PALCAM agar for enumeration. Inoculated XLD agar was incubated at 37°C for 24 h while inoculated PALCAM agar was incubated at 37°C for 48 h. Enumeration was performed after incubation.

The pre-enrichment and selective enrichment stages of the aforementioned standard analysis methods for samples contaminated with *S.* Typhimurium and *L. monocytogenes* were not performed, and no biochemical test was conducted.

Statistical analysis

All experiments were repeated three times independent of each other, and the means and standard deviations were determined. Statistical analyses were performed using SPSS/PC version 10.0 software. Statistical comparison between groups was performed using ANOVA (one-way variance analysis). Mean differences between the groups was performed using the Duncan test.

Results

In the present work, the antibacterial effects of four different decontaminants (slightly acidic electrolysed water, chlorine dioxide, calcium oxide, and nisin) and two different application methods (spraying and immersion) against *S.* Typhimurium and *L. monocytogenes* strains artificially inoculated on chicken breast meats were investigated. A statistically significant difference was obtained between *S.* Typhimurium and control. When the decontaminants were inspected separately, immersion method at day 0 for SAEWS and SAEWI groups was found to be more efficient with a reduction of 0.85 log, and the antibacterial effect persisted for the following days of the cold storage. In ClO_2S treatment, no difference was determined for the samples during the cold storage period, whereas ClO_2I application resulted in a significant difference at day 6 ($p < 0.05$). Similarly, CaOS application resulted in no difference between the samples during the cold storage period, whereas CaOI resulted in a difference at day 6 ($p < 0.05$). For nisin, both application methods had differences in samples collected throughout the cold storage, and

Table 2. The effects of four different decontaminants and two application methods againts *S.* Typhimurium.

Decontaminant	A				B
	Day 0	Day 1	Day 3	Day 6	
Control	6.37 ± 0.4^{a}	6.48 ± 0.4^{a}	6.70 ± 0.3^{a}	6.16 ± 0.4^{a}	6.24 ± 0.3^{a}
SAEWS	6.37 ± 0.2^{a}	6.23 ± 0.3^{a}	6.21 ± 0.3^{a}	5.21 ± 0.4^{b}	6.01 ± 0.6^{bc}
SAEWi	5.52 ± 0.1^{a}	5.85 ± 0.2^{a}	5.77 ± 0.8^{a}	5.28 ± 0.4^{a}	5.60 ± 0.4^{c}
ClO_2S	6.04 ± 0.7^{a}	6.0 ± 0.1^{a}	6.2 ± 0.5^{a}	5.60 ± 0.15^{a}	5.96 ± 0.5^{bc}
ClO_2i	6.2 ± 0.48^{ab}	6.2 ± 0.24^{ab}	6.7 ± 0.1^{a}	5.60 ± 0.46^{b}	6.17 ± 0.5^{ab}
CaOS	5.87 ± 0.3^{a}	5.97 ± 0.3^{a}	5.91 ± 0.6^{a}	5.33 ± 0.4^{a}	5.77 ± 0.4^{bc}
CaOi	5.78 ± 0.7^{ab}	6.16 ± 0.3^{ab}	6.61 ± 0.1^{a}	5.71 ± 0.3^{b}	6.06 ± 0.5^{ab}
NS	6.04 ± 0.1^{a}	5.78 ± 0.1^{ab}	6.05 ± 0.5^{a}	5.10 ± 0.5^{b}	5.74 ± 0.5^{bc}
Ni	5.71 ± 0.12^{a}	5.89 ± 0.09^{a}	6.32 ± 0.4^{a}	5.63 ± 0.69^{a}	5.89 ± 0.45^{bc}

A = Average results of decontaminant and application methods during the storage period, and
B = Statistical results of the control group and decontaminant and application methods.

the antibacterial effect persisted all the way through. NS and NI applications were found to have 1.06 and 0.53 log/g reduction as compared to the control group at the end of the storage period (6th day). As a result, the most effective decontaminant against *S.* Typhimurium was found to be the slightly acidic electrolysed water, whereas the most effective application method was determined to be the immersion method (Table 2).

There was a statistically significant difference between four different decontaminants, two application methods, and the control group for chicken breast meat samples artificially inoculated with *L. monocytogenes*. Nisin was determined to be the most effective decontaminant for *L. monocytogenes* in both application methods. At day 0, NS and NI yielded a reduction of 2.82 and 2.76 log/g as compared to the control group. Furthermore, nisin has sustained its antibacterial effects during the cold storage period. The weakest antibacterial effect on *L. monocytogenes* was achieved by immersion in slightly acidic electrolysed water, contrary to its success against *S.* Typhimurium (Table 3).

Discussion

Various contaminations may occur at various stages of chicken meat production (slaughter, processing, packaging, distribution, and preparation). Microbial contamination of chicken meat causes great economic concern for producers, and serious problems for public health (Petrou *et al.,* 2002). Various decontaminants and decontamination methods are being applied in order to reduce such contaminations to tolerable levels or to completely eliminate them (Özbay and Sarıcoban, 2015). Chemical decontamination processes exert their bactericidal effects by targeting their cell membranes, cell content, or physiological functions (Loretz *et al.,* 2010). Various studies suggested that it is possible to use certain decontaminants to significantly reduce the initial microbial load. Al-Holy and Rasco (2015) have determined that acidic EW applied on beef, chicken, and fish meat reduced *S.* Typhimurium count by 1.5 - 1.6 log. On the other hand, Fabrizio *et al.* (2002) reported that EW spraying and immersion methods applied on chicken carcasses resulted in 0.87 and 0.83 log reduction in day 0, and 1.85 and 0.98 log reduction at the end of day 7, respectively. The findings of these two studies were found to be similar in comparison to the findings obtained in the present work for SAEWI. These researchers have also reported a reduction of 4 log when neutral EW washing is applied after acidic EW immersion. This shows that this type of application is more effective than the method used in the present work.

Besides these, Çil *et al.* (2012) performed a study in which they showed that EW exerted 2 log reduction in chicken meat at day 0, but its effect gradually diminished during the cold storage period. This can be explained by the organic matters present in the chicken meat used as study material, primarily the proteins, entering to fast reactions with the free chlorine available and reducing the antibacterial effect of the EW. Differing from the present work, some other studies reported that EW had a stronger effect over *Salmonella* spp.; Northcutt *et al.* (2007) reported that washing broiler carcasses with EW reduced *Salmonella* spp. by 2.7 log, and Mansur *et al.* (2015) reported that SAEW obtained with 0.5% fumaric acid reduced *S.* Typhimurium by 2.5 log. This difference might be due to the use of acidic EW or EW obtained in combination with organic acids like the fumaric acid.

As for the studies performed on *Salmonella* spp. to determine the antibacterial effects of ClO_2,

Table 3. The effects of four different decontaminants and two application methods against *L. monocytogenes*.

Decontaminant	A				B
	Day 0	Day 1	Day 3	Day 6	
Control	5.54 ± 0.1^{b}	5.58 ± 0.2^{b}	5.67 ± 0.2^{ab}	6.14 ± 0.1^{a}	5.7 ± 0.34^{a}
SAEWS	5.14 ± 0.2^{b}	5.12 ± 0.2^{b}	5.14 ± 0.2^{b}	5.89 ± 0.2^{a}	5.32 ± 0.5^{ab}
SAEWi	4.87 ± 0.1^{b}	5.14 ± 0.2^{b}	5.65 ± 0.1^{a}	6.11 ± 0.2^{a}	5.44 ± 0.5^{ab}
ClO_2S	4.79 ± 0.1^{b}	5.03 ± 0.1^{b}	4.97 ± 0.2^{b}	6.20 ± 0.2^{a}	5.25 ± 0.6^{b}
ClO_2i	4.83 ± 0.4^{b}	5.14 ± 0.2^{ab}	5.31 ± 0.3^{ab}	5.95 ± 0.1^{a}	5.31 ± 0.6^{ab}
CaOS	5.07 ± 0.2^{bc}	4.96 ± 0.1^{c}	5.50 ± 0.9^{b}	6.22 ± 0.2^{a}	5.44 ± 0.6^{ab}
CaOi	4.90 ± 0.5^{b}	4.85 ± 0.1^{b}	5.63 ± 0.4^{a}	6.03 ± 0.1^{a}	5.35 ± 0.6^{ab}
NS	2.72 ± 0.1^{a}	2.79 ± 0.1^{a}	2.73 ± 0.2^{a}	2.98 ± 0.1^{a}	2.80 ± 0.2^{c}
Ni	2.78 ± 0.1^{b}	2.79 ± 0.1^{b}	2.78 ± 0.1^{b}	3.13 ± 0.1^{a}	2.87 ± 0.2^{c}

A = Average results of decontaminant and application methods during the storage period, and B = Statistical results of the control group and decontaminant and application methods.

Alonso-Hernando *et al.* (2015) reported that 50 ppm ClO_2 inoculated into skin-free chicken drumsticks reduced *S.* Enteritidis by 0.59 log. On the other hand, in another study performed by Pohlman (2002) using another material, the results indicate that the application of CT (200 ppm chlorine dioxide and 10% trisodium phosphate) in minced meat had no effect on *S.* Typhimurium. The findings of these studies are similar to our findings. Differing from the present work in that regard, Xu (2005) reported that chicken breast meats contaminated with 10^7 CFU/g level treated with 40 ppm ClO_2 for 6 min yielded 0.9 log reduction in pathogenic count. The difference with the present work in that regard might be due to the treatment duration with the decontaminant.

The literature survey performed as part of the present work has revealed that no other studies have been done on the antibacterial effects of calcium oxide and nisin on *Salmonella* spp. The present work demonstrated that calcium oxide is the second most effective decontaminant after EW in terms of its effects on *S.* Typhimurium. Nisin, on the other hand, was revealed to have the weakest antibacterial effect as a decontaminant on *S.* Typhimurium. This may be explained by the fact that nisin has a weak antibacterial effect against Gram-negative bacteria in general.

In other study performed to investigate the effectiveness of EW on *L. monocytogenes*, Al-Holy and Rasco (2105) reported that acidic oxide EW applied on beef, chicken, and fish meat reduced bacterial count by 1.1 to 1.3 log, and Mansur *et al.* (2015) reported that slightly acidic EW obtained using 0.5% fumaric acid reduced *L. monocytogenes* count by 3.0 log approximately. In the present work, *EW* was found to have no significant antibacterial effect against *L. monocytogenes*. This can be due to *L. monocytogenes* being resistant to disinfectants, its ability to survive in various types of environmental conditions, and the study material being the chicken meat parts, which could provide a protection ground for pathogens due to its irregular surface structure.

As for the studies performed to determine the antibacterial effects of chlorine dioxide on *L. monocytogenes*, Hong *et al.* (2008) reported a reduction of 0.61 and 1.93 log CFU/g in chicken breast and drumstick meats at day 0, respectively, when they inoculated 8 - 9 logs CFU/g of *L. monocytogenes*. On the other hand, they also reported that the antibacterial effects of chlorine dioxide diminished over the course of the cold storage period. In another study, Alonso-Hernando *et al.* (2015) reported that the application of 50 ppm ClO_2I method for 15 min reduced the *L. monocytogenes* inoculated into chicken drumstick meat by 0.31 log CFU/g at day 0. The findings of both studies are similar to that of our own where ClO_2S and ClO_2I methods achieved a reduction of 0.71 and 0.47 log CFU/g reduction, respectively, and the antibacterial effects of the decontaminant also diminished over time.

There are limited studies available on the antibacterial effects of CaO solutions against *L. monocytogenes*. In one of these studies, Bae *et al.* (2006) reported that CaO added into water for disinfection with a rate of 0.05% reduced *L. monocytogenes* by 1.44 log CFU/mL. Cagri-Mehmetoglu (2011) reported that chicken wings inoculated with *L. monocytogenes* and then treated with 0.1 and 0.5% CaO mixtures saw a reduction of pathogenic count of 3.8 and 4.4 log CFU/g, respectively, and no significant change was observed during storage. Based on the day 0 results in the present work, CaOS and CaOI applications reduced the *L. monocytogenes* count by 0.47 and 0.64 log CFU/mL, respectively. The difference between the previous studies and the present one was attributed to the difference in materials used, concentration of the disinfectant, organic compounds present in the medium, and the contact surface contributing to a stronger bactericidal effect.

On the effectiveness of nisin as a decontaminant for *L. monocytogenes*, Wan Norhana *et al.* (2012) reported that it reduced the pathogenic count by 0.95 log in shrimps; Mahadeo and Tatini (1994) reported a reduction of 1 log in turkey meat; Zhang and Mustapha (1999) reported a reduction of 2.01 log in cattle meat; Ariyapitipun *et al.* (1999) reported a reduction of 1.64 log in vacuum-packed raw cattle beef; and El-Khateib (1993) reported a reduction of 0.9 log in pieces of meat. Day 0 results of the present work for NS and NI applications against *L. monocytogenes* revealed a result of 2.82 and 2.76 log CFU/g, respectively. It appears that the present work exhibited a stronger effect for nisin as compared to the other studies in the literature, in terms of antibacterial effect. This difference might be due to the pH of the solution, the producer bacterial strains of nisin, and the variations in application methods used. The present work also revealed that nisin had a stronger antibacterial effect against *L. monocytogenes* as compared to against *S.* Typhimurium. This may be due to nisin having a stronger efficiency against Gram-positive bacteria.

Conclusion

Although chlorine dioxide is the commonly

used decontaminant in poultry animal slaughterhouses, the results of the present work indicated that electrolysed water had a weak antibacterial effect on *L. monocytogenes*, but it had a stronger antibacterial effect against *Salmonella* strains, which are the most important pathogens for poultry meats. Furthermore, electrolysed water is cheap, safe for the environment, has no toxicity, and applicability on foods and food contact surfaces grants it strong advantages. Due to these properties, we have concluded that the use of this decontaminant in poultry animal slaughterhouses is appropriate, particularly for control of *Salmonella* species.

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