Efficacy of chlorine dioxide against *Listeria monocytogenes* in brine solutions

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Introduction

Chlorine dioxide (ClO_2) is an antimicrobial recognized for its disinfectant properties since the early 1900's (EPA, 2005). This compound effectively kills microorganisms including bacteria, viruses or fungi on inanimate objects, foods, and other surfaces (EPA, 2005). Chlorine dioxide can be generated in a gas or liquid form and smells like chlorine bleach (EPA, 2005). In 1967, the Environmental Protection Agency (EPA) first registered the liquid form of chlorine dioxide for use as a disinfectant and sanitizer and in 1988, EPA registered chlorine dioxide gas as a sterilant (EPA, 2005). The mode of action of chlorine dioxide against microorganisms is to disrupt transport of nutrients across the cell wall (EPA, 2005). ClO_2 is considered more effective against microbes than aqueous chlorine solutions (Cutter and Dorsa, 1996).

Chlorine dioxide is generated by mixing either sodium chlorite with another "reactive" chemical – such as an acid - to produce chlorine dioxide in an aqueous (liquid) or gaseous state (EPA, 2005). When compared with chlorine solutions, ClO_2 efficacy is not affected by pH or presence of ammonium (Kim et al., 1999a). The resulting aqueous mixture can be applied to foods as a fine spray or dip. Aqueous chlorine dioxide also can be applied to hard surfaces with a sponge or mop (EPA, 2005). Chlorine dioxide also has been proven safe and effective when used for water disinfection (Kim et al., 1999; 40 CFR 141.2 and 141.72; AMI petition, 2005).

In 1995, chlorine dioxide was approved as a food additive in poultry chiller water (Kim et al., 1999a). Today, the compound is permitted in a variety of foods for human consumption (AMI petition, 2005). Chlorine dioxide at levels of 3 ppm or less can be used as an antimicrobial agent in water used for the chilling of poultry, when used to wash fruits and vegetables, applied to red meat carcasses, seafood, as well as to parts and organs (21 CFR 173.325; AMI petition, 2005). Additionally, generation of aqueous chlorine dioxide has been demonstrated to be safe for plant workers, federal inspection personnel, and consumers (21 CFR 173.325; AMI petition, 2005).

The meat industry is interested in using chlorine dioxide as an antimicrobial in brine and chilling water systems used for cooling of ready-to-eat (RTE) meat and poultry products. According to a recent AMI petition (2005), “the low levels (3 ppm) proposed for use for brine and chilling water systems used in processing of ready-to-eat meat and poultry products are similar to those approved for other food applications. A regulatory limit of $\leq$ 3 ppm residual chlorine dioxide in the brine or chilling water is the same level currently specified by 21 CFR 173.300. The 3 ppm level represents the concentration of chlorine dioxide remaining in the brine or chilling water after contact with the food, rather than an upper limit on the amount of chlorine dioxide introduced into the system. This requires a dosage of chlorine dioxide modestly in excess of 3

ppm to ensure that enough oxidant is available to react with reducing substances and to still leave a residual level of 3 ppm of chlorine dioxide."

Given the above information and lack of published studies, the efficacy of chlorine dioxide against *Listeria monocytogenes* in brine solutions has not been investigated. While a number of studies have focused on the effect of ClO_2 for reducing microbes on meat or poultry (Thiessen et al., 1984; Villarreal et al., 1990; Cutter and Dorsa, 1995; Pohlman, et al., 2002; and Stivarius, et al., 2002), seafood (Kim et al., 1999a), only one other study has investigated survival of *Listeria monocytogenes* in brine solutions (Gailey et al., 2003). In this study, researchers demonstrated that *L. monocytogenes* can survive in brine solutions at varying pH levels and with different concentrations of chlorine (Gailey et al., 2003). These findings demonstrate a need to identify potential interventions to control, inhibit, and prevent the growth of *L. monocytogenes* in brine solutions used for chilling of RTE meat products. Given the limited information to date**,** the objective of this study is to determine the efficacy of the 3 ppm chlorine dioxide against *Listeria monocytogenes* in clean and spent brine solutions.

Materials and Methods

Culture storage, inocula preparation, enumeration, and enrichment procedures:
Listeria monocytogenes Scott A (ATCC 14589; American Type Culture Collection, Manassas, VA), and four strains of *L. monocytogenes* from recent RTE outbreaks (epidemic clones I and II; CDC, B. Swaminathan, personal communication) were obtained from the culture collection at the Pennsylvania State University Department of Food Science and stored at –70°C in tryptic soy broth (TSB; Difco Laboratories, Detroit, MI) containing 10% glycerol. Prior to experiments, *L. monocytogenes* was propagated twice in TSB at 37°C for 24 h. The cultures were maintained on tryptic soy agar (TSA; Difco Laboratories, Detroit, MI) slants and transferred weekly to maintain viability. One loopful of *L. monocytogenes* was obtained from TSA slants and aerobically incubated in 9 mL TSB for 24 h at 37°C. Following incubation, 1 ml of the overnight culture was aseptically transferred to sterile dilution bottles containing 99 ml of TSB. After 10 seconds of agitation, the inoculum was incubated for 18 h at 37°C to obtain 9 $\log_{10}$ CFU/ml mid-stationary phase cells. One ml of this culture was centrifuged at 5,000 x g for 10 min (Gailey et al 2003), after which the supernatant was decanted, and the cells were resuspended in 1 ml of a sterile saline solution (0.07% of NaCl; BDH, West Chester PA). After centrifuging again, the supernatant was removed and resuspended again in 1 ml of sterile saline solution. For experiments #1, 2, and 3, inocula prepared in this manner were added to the 99 ml of brine solutions to obtain 7 $\log_{10}$ CFU/ml or serially diluted and added to 99 ml of brine to obtain 5 $\log_{10}$ CFU/ml.

All samples generated in this study that were subjected to treatments with chlorine dioxide were enumerated by serially diluting in 0.1% peptone water plus 0.03% sodium thiosulfate (16), spiral plating (Spiral Biotech, Norwood, MA; Experiment #2a) or direct plating 1 ml in duplicate onto TSA plates (Experiments 1, 2b, 3, and 4), and incubating for 48 h at 37ºC. Plates were enumerated manually.

Preliminary inoculation experiments (Experiment #1)

In order to determine the effect of brine solutions on *L. monocytogenes*, in the absence of chlorine dioxide, a series of preliminary experiments were conducted. Calcium and sodium chloride (VWR, West Chester PA) solutions (99 ml) at 10 and 20%, were made using deionized water, autoclaved to ensure sterility, and chilled to 0°C using a circulating water bath. The pH of the brine was adjusted to 7.0 with HCl (0.1 N) prior to inoculation with the pathogen. Overnight cultures of the pathogen were washed as described above and added to 99 ml of individual brine solutions to obtain approximately 7 $\log_{10}$ CFU/ml. Inoculated solutions were stored at 0°C for up to 24 h and resulting populations determined every 2 h for 8 h, and again at 12 and 24 h by sampling, diluting, and enumerating on TSA plates as described above. The experiment was performed in triplicate. D-values were calculated from this experiment.

Generation of chlorine dioxide

Chlorine dioxide (ClO_2) was generated in tap water using a Halox System (Bridgeport, CT) located in the Department of Food Science (Penn State University). In order to ensure that the ClO_2 concentration remained stable in brine solutions or chill water, ClO_2 was generated at approximately 929 ppm, stored in a dark container, and chilled immediately to 4°C. Immediately after generation and prior to experiments, the stock solution was measured for chlorine dioxide concentration with a Photometer Chlordioxmeter 1000 (Palintest, Gateshead UK) that utilizes the DPD method (N, N-diethyl-p-phenylenediamine) for detection.

"Clean" brine solutions and inoculation with L. monocytogenes (Experiment #2)

Washed cultures of *L. monocytogenes* were added to 99 ml of sterile, chilled brine solutions (10 and 20% sodium and calcium chloride solutions) to achieve a final concentration of 5 (Experiment #2a) or 7 $\log_{10}$ CFU/ml (Experiment 2b). Immediately after inoculation with the pathogen into the brine solutions, a sample was taken for enumeration purposes. Then, a stock solution of 929 ppm chlorine dioxide was added to the test solutions such that the final concentration in the inoculated brines was approximately 3 ppm. Sterile, deionized water solutions chilled to 0°C using a circulating water bath were used as controls in these experiments. After treatment with chlorine dioxide, brine solutions were held for up to 10 min for Experiment 2a and up to 180 s for Experiment 2b.

Since a precipitant formed during the detection process (due to an unknown chemical reaction), it was difficult to obtain residual chlorine dioxide readings using the Photometer Chloridioxmeter from brine samples. So, a determination of chlorine dioxide dosage was made in the brine solutions by adding an equivalent amount of the stock solution of chlorine dioxide into deionized water and taking measurements with the Photometer Chloridioxmeter.

In Experiment 2a, samples were obtained every 2 min by aseptically removing 1 ml of inoculated, treated brine and serial diluting the solution in 0.07% saline solution containing 0.03% sodium thiosulfate for neutralization of the chlorine dioxide, followed by spiral plating on TSA plates as described previously. The experiment was performed in triplicate. For samples were no pathogen was detected by spiral plating, samples also were subjected to enrichment procedures as follows. Briefly, one milliliter of the individual samples was transferred to 9 mL of University of Vermont Modified (UVM; Difco Laboratories, Detroit, MI) pre-enrichment broth. Following aerobic incubation for 24 ± 2 h at 37 ± 2^{o}C, 1ml UVM broth was transferred to 9 ml

of Fraser broth (Difco Laboratories, Detroit, MI) and aerobically incubated at 37 ± 2°C for 24 ± 2 h. Fraser broth samples were spread plated in duplicate onto MOX, inverted and aerobically incubated at 37 ± 2°C for 24 ± 2 h. Presumptive positive MOX plates displaying typical colonies with black zones were picked and confirmed as *Listeria* spp. using TECRA visual immunoassay of Listeria (TECRA International, Australia).

In Experiment 2b, samples were obtained every 30 sec by aseptically removing 1 ml of inoculated, treated brine and serial diluting the solution in 0.1% peptone water containing 0.03% sodium thiosulfate for neutralization of the chlorine dioxide, followed by direct plating 1 ml on TSA plates in duplicate as described previously. The experiment was performed in triplicate. D-values also were calculated from these experiments.

Effect of cross protection on L. monocytogenes (Experiment #3)

Results from Experiment 2b demonstrated increased survivability of *L. monocytogenes* when suspended in 20% calcium chloride and treated with 3 ppm chlorine dioxide. However, it was not clear if there was enhanced resistance of the pathogen to the compound following exposure to 20% calcium chloride or if the compound was being depleted by the concentrated brine solution. To address this issue, cells were pre-treated in chilled brine solution or water (controls), transferred to chilled sterile distilled water, and then treated with chlorine dioxide as follows. An overnight culture of *L. monocytogenes* was centrifuged at 5,000 x g for 10 minutes, supernatant decanted, and cells resuspended in 1 ml of chilled water, 10% sodium chloride, 20 % sodium chloride, 10% calcium chloride, or 20% calcium chloride for 2 min at 0°C. After centrifugation again, the supernatant was removed and cells were resuspended in 1 ml of sterile deionized water and added to 99 ml of chilled distilled water to obtain approximately 7 $\log_{10}$ CFU/ml. The stock solution of chlorine dioxide was added to the inoculated water solutions to obtain approximately 3 ppm. All samples were incubated for 30 sec and resulting bacterial populations serial diluted and plated in duplicate on TSA as described above. The experiment was performed in triplicate.

"Spent" brine solutions and inoculation with L. monocytogenes (Experiment #4)

Using a similar format to Experiment #2b, spent brine solutions (from hot dogs and hams treated with brine solutions) were obtained directly from industry and stored at 4°C for approximately 2 months until experiments were conducted. During that time, compositional analyses were performed on the solutions. Spent solutions from hams contained 3% organic material with a final concentration of ~6% sodium chloride. Spent solutions from hot dogs contained 1% organic material with a final concentration of ~16% sodium chloride.

Prior to experiments, spent brine solutions were transferred to sterile test tubes and chilled to 0°C in a circulating water bath. *L. monocytogenes* was inoculated into the spent brine solutions to achieve 7 $\log_{10}$ CFU/ml. After inoculation with the pathogen, the stock solution of chlorine dioxide was added to the test solutions such that the final concentration was approximately 3 or 30 ppm, and held at 0°C. Samples were taken every 30 sec for up to 180 sec and enumerated as described above. Approximate chlorine dioxide levels were determined using the Photometer Chlordioxmeter 1000 in all solutions and results recorded. Each trial was repeated in triplicate.

Calculation of D-Values
The inactivation rate was measured by determining the decimal reduction time (D-value) calculated from the average of the inverse slope of the three survival curves of every treatment, including the control. The curves were obtained by plotting $\log_{10}$ values versus the corresponding time intervals and using linear regression to determine the slope.

Determination of water activity
Water activity of brine solutions, a_w, was measured with a Decagon CX-1 (Decagon Devices, Pullman, WA), calibrated, and operated according to the manufacturer. The measurement temperatures were between 17.1 and 18.1°C. The experiment was performed in triplicate.

Statistical analysis
Microbial populations obtained from TSA agar plates were enumerated manually to obtain CFU/ml, counts averaged from duplicate plates, and converted to $\log_{10}$ CFU/ml. The converted data was analyzed using Statistical Software, release 15.00 (Minitab Inc, State College PA). Comparisons were done using one-way ANOVA followed by Tukey's multiple comparison test. The slope of the curves was obtained using linear regression, and the D value was obtained using the inverse slope of the equation.

Results and Discussion

Several research reports have focused on the use of interventions to control pathogens in poultry chilling water or chilling brines. Only a few of have addressed the use of chlorine dioxide as an antimicrobial to inhibit pathogens in these systems. In this study, we demonstrate the feasibility and limitations of using chlorine dioxide to control *Listeria monocytogenes* in brine solutions commonly used by the ready-to-eat meat industries.

Experiment #1:
The characteristics of the individual brine solutions used in this study are presented in TABLE 1. For this first experiment, populations of *L. monocytogenes* were monitored periodically for up to 24 hours following inoculation directly into brine solutions held at 0ºC. This experiment was performed as a means to determine the survivability of the pathogen without the effect of chlorine dioxide. While the addition of the pathogen to 10, 20% sodium chloride or 10% calcium chloride did not affect a significant reduction over 8 h, there was an immediate lethal effect (reduced ~ 1.5 $\log_{10}$ CFU/ml) when *L. monocytogenes* was added to 20% calcium chloride (FIGURE 1). After 12 and 24 h at 0ºC, the pathogen was reduced approximately 3 $\log_{10}$ CFU/ml in 20% calcium chloride (data not presented). These findings support other studies where *L. monocytogenes* was found to be halotolerant, including one study that reported the survival of *L. monocytogenes* for up to 30 days at –12°C in 20% sodium chloride (Miller, et al., 1997). Another study demonstrated the survival of the pathogen in commercial cheese brines after 259 days (Larson et al., 1999). Interestingly, our findings are the first report addressing the survival of the pathogen in calcium chloride brines.

The average D-values for brine solutions inoculated with *L. monocytogenes* were 597, 466, 294, 78 and 11 h for control, 10% sodium chloride, 20% sodium chloride, 10% calcium chloride, and 20% calcium chloride, respectively. Of the solutions, the D-value obtained using the 20% calcium chloride brine solutions demonstrated a significant difference ($p<0.05$), as compared to

the control and the two sodium chloride brine solutions; but no significant difference was observed between the 10% and 20% calcium chloride brine solutions.

Experiments #2a and 2b:

In these experiments, chilled brine solutions inoculated with *L. monocytogenes*, were treated with ~3 ppm chlorine dioxide (as determined by adding an equivalent amount to water). The addition of chlorine dioxide affected immediate and significant reductions in pathogen populations, regardless of initial inocula level.

When inoculated with 5 $\log_{10}$ CFU/ml for up to 10 min (Experiment 2a), pathogen populations were reduced to non-detectable levels by spiral plating methods within 2 minutes (data not presented). Only after enrichment procedures was the pathogen detected in some samples (data not presented). Given that there was an immediate and sustained reduction in the pathogen following a long exposure time to chlorine dioxide, additional experiments were conducted within 90 seconds (Experiment 2b). Additionally, D-values from samples with low levels (<5 $\log_{10}$ CFU/ml) of the pathogen could not be calculated. So, all remaining experiments were performed using 7 $\log_{10}$ CFU/ml of the pathogen.

In Experiment 2b, populations of *L. monocytogenes* were reduced >3.5 $\log_{10}$ CFU/ml when sampled for up to 90 sec in 10% sodium chloride, 20% sodium chloride, and 10% calcium chloride brine solutions (FIGURE 2). However, the addition of approximately 3 ppm chlorine dioxide to a solution of 20% calcium chloride inoculated with the pathogen only reduced *L. monocytogenes* <2 $\log_{10}$ CFU/ml. Interestingly, a precipitate was formed when 3 ppm chlorine dioxide was added to 20% calcium chloride brine solutions. It was hypothesized that the precipitate and/or brine solution interfered with the antimicrobial activity of the compound by 1) affording some protection to the pathogen or 2) binding up the available chlorine dioxide so there was not a sufficient amount to inhibit the pathogen.

The average D-values for brine treatments inoculated with *L. monocytogenes* and treated with 3 ppm chlorine dioxide were 50.9, 26.2, 23.5, 23 and 21.9 sec for control, 10% sodium chloride 20% sodium chloride 10% calcium chloride, and 20% calcium chloride, respectively. The D-value obtained using the 20% calcium chloride demonstrated a significant difference ($p<0.05$), as compared with the other treatments, including the control.

Experiment #3.

As indicated earlier, *L. monocytogenes* suspended in 20% calcium chloride and treated with 3 ppm chlorine dioxide was not reduced to the same extent as other brine solutions. While there are reports from the water treatment industry that chlorine dioxide can oxidize both iron and manganese, there is no research focusing on the role of inorganic compounds, potential interference in brine chilling, or the effects of these compound on chlorine dioxide performance.

In two recent studies, chlorine dioxide reacted with soluble forms of iron and manganese to form precipitates that were removed through sedimentation and filtration (Henderson et al., 2001). Therefore, the potential exists that soluble minerals can reduce chlorine dioxide availability in solution. Other studies have addressed similar issues. In one report, adapting *Listeria* cells to mildly acidic conditions rendered cultures more resistant to relatively high (25-30%) carbon

dioxide atmospheres (Francis and O'Beirne, 2001). In another study, the prior adaptation to sub-lethal levels of bile acids or heterologous stresses, such as acid, heat, salt, or sodium dodecyl sulfate, significantly enhanced bile resistance (Begley et al., 2002). And finally, exposure to diverse stresses including starvation and treatment with hydrogen peroxide, ethanol, and low pH treatments caused increases in the thermotolerance of *L. monocytogenes* (Lou and Yousef, 1996). Low sodium chloride concentration (2 to 3.5%) has also been shown to have a protective effect against the food preservative nisin at low temperatures (De Martinis et al., 1997). As such, this issue should be considered during the application of chlorine dioxide in brine solutions.

In this experiment, cells were exposed to brine solutions, followed by transfer to water, and subsequently treated with 3 ppm chlorine dioxide. Pre-exposed *L. monocytogenes* treated with chlorine dioxide for 30 sec were reduced significantly (approximately 3 $\log_{10}$ CFU/ml; FIGURE 3). These results suggest that prior exposure to the brine solutions does not offer any protection to the pathogen. But rather, the salt solution appears to be binding to, interfering with, or diminishing the oxidizing capacity of the chlorine dioxide such that the compound is not active against the cells.

Experiment #4.

In this experiment, the efficiency of chlorine dioxide in spent brine was evaluated. As indicated in FIGURE 4, levels of *L. monocytogenes* inoculated into both spent brine solutions and treated with 3 or 30 ppm chlorine dioxide were not reduced. This finding suggests that the spent brines, with as little as 1% organic material, appear to have a high chlorine dioxide demand, due in part to the level of organic material (protein, fat, etc.) suspended in the brine. The chlorine dioxide appears to be binding to the organic material and may not be available to affect reductions against the pathogen. Since no significant reductions were observed, D-values for this experiment could not be calculated.

Based on these findings, the following recommendations should be considered. Before chlorine dioxide is selected for use as a primary disinfectant in spent brine systems, an oxidant demand study should be completed. If the oxidant demand is significant, chlorine dioxide should not be used as a disinfectant in these solutions. Filtration could be employed to remove most of the organic material from spent brine prior to disinfection to improve the efficacy of chlorine dioxide against pathogens in solution if companies choose to reuse the brine solutions. However, very little, if any research has been conducted with filtration to reduce organic loads and improve efficacy of antimicrobials.

Conclusions and Recommendations

This study has demonstrated that *Listeria monocytogenes* is able to survive in brine solutions composed of 10 and 20% sodium chloride, and 10% calcium chloride after 8 h at 0ºC. These findings are in agreement with other published research studies. However, *L. monocytogenes* was reduced ~1.2 log10 CFU/m when suspended in 20% calcium chloride brine solutions after 8 h at 0ºC. Approximately 3 $\log_{10}$ CFU/ml reduction was observed after 24 h. This information has not been reported previously.

In 10 and 20% sodium chloride and 10% calcium chloride brine solutions, a 3 ppm chlorine dioxide treatment for 90 sec at 0ºC resulted in approximately 4 $\log_{10}$ CFU/ml reduction in *L. monocytogenes.* However, in 20% calcium chloride solutions, *L. monocytogenes* was reduced approximately 2 $\log_{10}$ CFU/ml in 90 sec. It should be noted that a precipitate was observed during experiments with 3 ppm chlorine dioxide in 20% calcium chloride.

Subsequent experiments demonstrated that previous exposure of *L. monocytogenes* in brine solutions does not impart protection or enhanced resistance against chlorine dioxide. It appears that the 20% calcium chloride affects the activity of chlorine dioxide by potentially binding up the compound, interfering with activity, or diminishing the oxidizing capacity. As such, the use of calcium chloride brine solutions at a 20% concentration should be carefully evaluated for use in combination with 3 ppm chlorine dioxide.

And finally, 3 ppm chlorine dioxide was not effective for reducing *L. monocytogenes* in spent brine solutions from hot dogs and hams due to the presence of organic and possibly inorganic material. Interventions, such as filtration of spent brine solutions prior to disinfection with chlorine dioxide, should be investigated to improve the efficacy of the compound against *L. monocytogenes.*

Suggestions for Future Research

- Determine the mechanisms of inactivation of 20% calcium chloride against *L. monocytogenes.*
- Identify chemicals formed during treatments between sodium chloride and calcium chloride solutions when treated with chlorine dioxide.
- Compare chlorine dioxide with other antimicrobials in brine solutions to inhibit *L. monocytogenes.*
- Conduct filtration experiments or other interventions to improve efficacy of antimicrobial solutions in spent brine solutions.
- Determine residual chlorite levels in brines and on ready-to-eat meat surfaces following treatments with chlorine dioxide.

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Table 1. Characteristics of brine solutions used in experiments 1-4.

Solution	Initial pH	pH after 1.5 min	pH reduction	Water activity	Temperature of solution	Miscellaneous
10% Sodium chloride	7	6.66	0.34	0.979	17.9	
20% Sodium chloride	7	6.74	0.26	0.921	17.1	
10% Calcium chloride	7	6.72	0.28	0.997	17.8	
20% Calcium chloride	7	6.85	0.15	0.968	17.1	
Spent brine from hams	5.93	NA	NA	0.958	17.5	6% sodium chloride; 3% organic material
Spent brine from hot dogs	6.36	NA	NA	0.999	18.1	16% sodium chloride; 1% organic material

<u>FIGURE 1.</u> Experiment #1: Survival of *Listeria monocytogenes* in brine solutions (10 or 20 % sodium [NaCl] or 10 or 20% calcium chloride [CaCl2]) stored at 0°C for up to 8 h. Each data point represents the mean of three experimental replications.

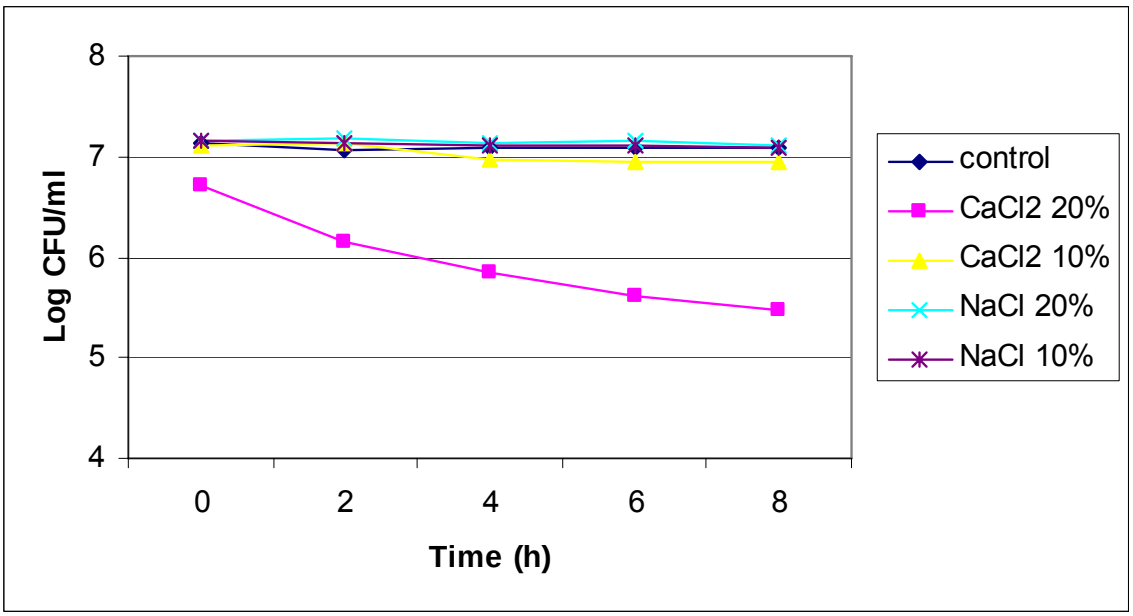
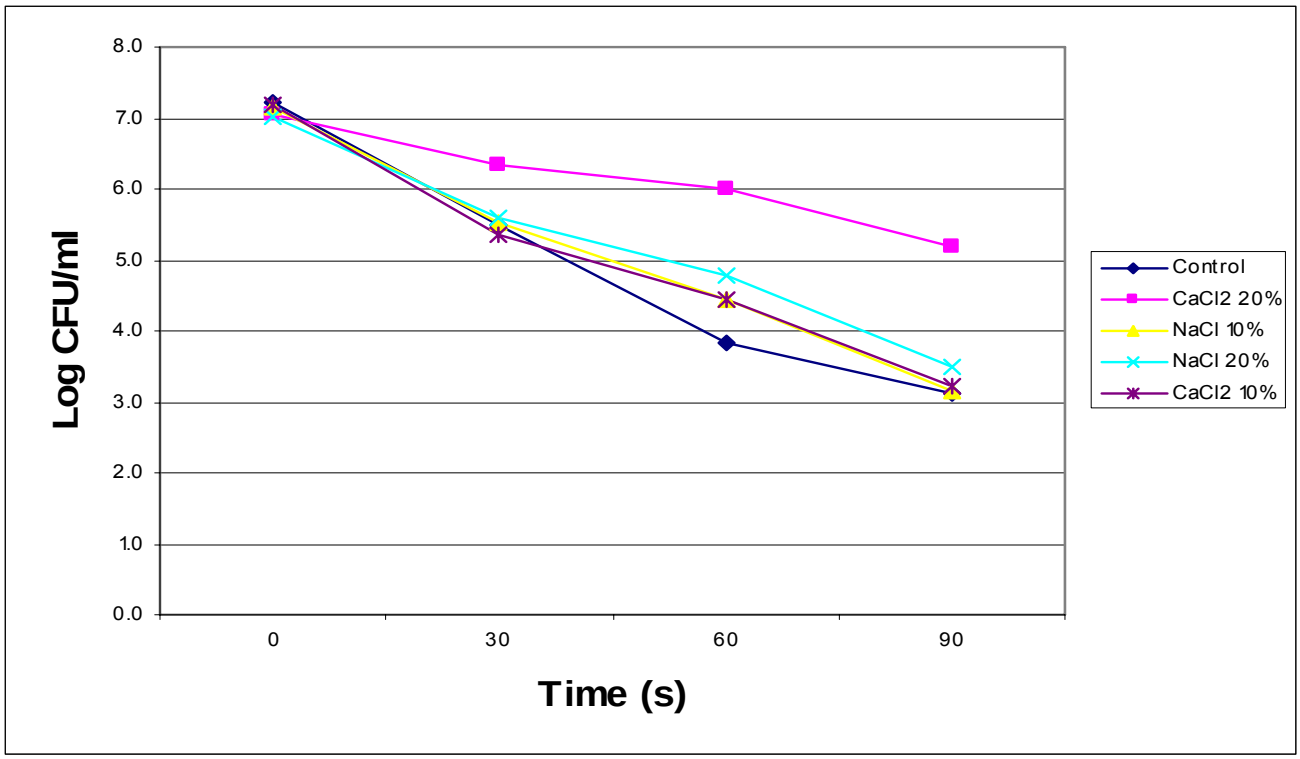
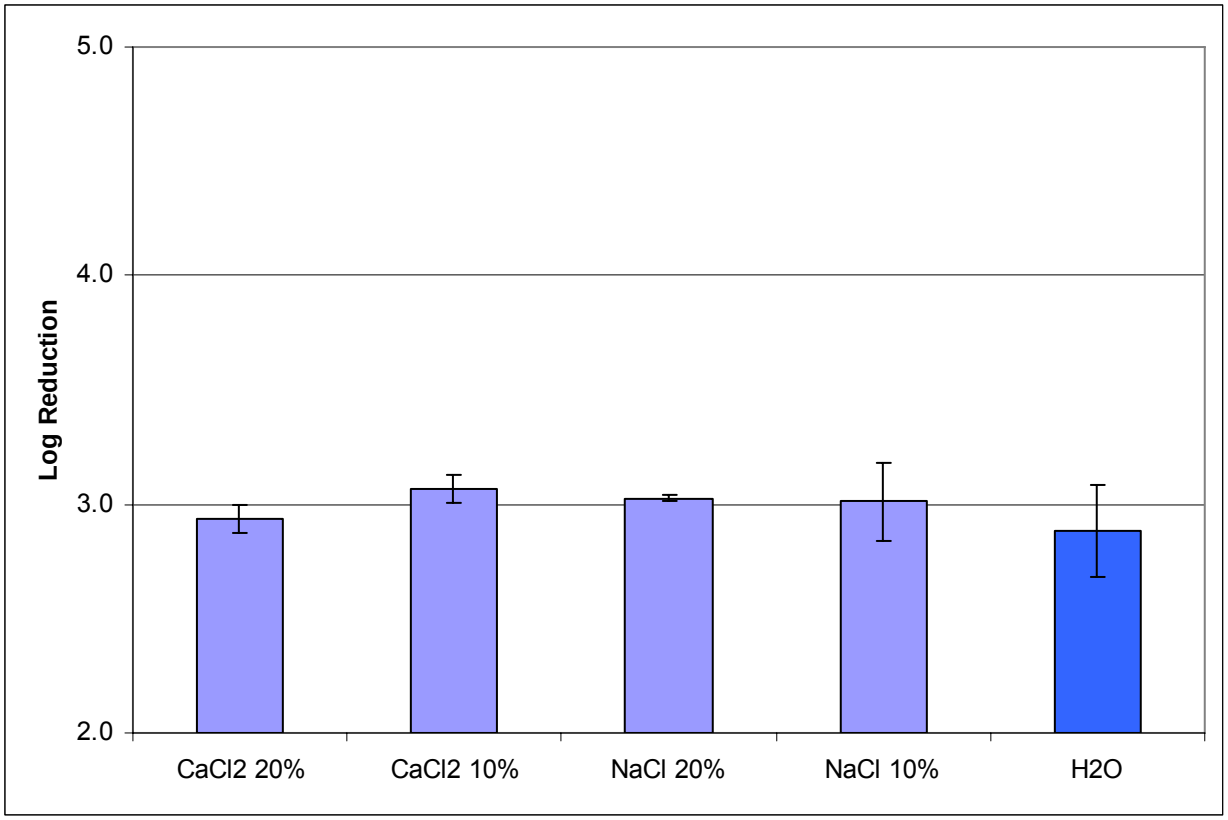


FIGURE 2. Experiment #2: Populations of *Listeria monocytogenes* in various brine solutions (10 and 20% CaCl2; 10 and 20% NaCl), chilled to 0°C and treated with 3 ppm chlorine dioxide for up to 90 sec. Each data point represents the mean of three experimental replications. 0-time is the initial count with no chlorine dioxide added.



<u>FIGURE 3.</u> Experiment #3: Average log reduction (log_{10} CFU/ml) of *Listeria monocytogenes* following a 2min exposure to distilled water [D water], brine solutions (10% sodium chloride [NaCl 10%], 20% sodium chloride [NaCl 20%], 10% calcium chloride [CaCl2 10%], 20% calcium chloride [CaCl2 20%] at 0ºC, followed by transfer to distilled water, and treatment with 3 ppm chlorine dioxide for 30 sec. Each bar represents the average log_{10} CFU/ml from three experimental replications.



<u>FIGURE 4.</u> Reduction of *Listeria monocytogenes* in spent brine at 0°C after adding the equivalent of 3 or 30 ppm of chlorine dioxide. S1=Spent brine from hot dogs; S2= spent brine from hams.