Efficacy of 1100 ppm PAA (Perasan MP-2®) and 335 ppm Br_2 (HB2) On *Salmonella* Heidelberg-Inoculated Chicken Halves

Background

The contamination of food products by pathogenic organisms such as *Salmonella spp.* is an on-going problem that is addressed within the processing plant using antimicrobial products. *Salmonella enterica enterica* is a subspecies of *Salmonella enterica. Salmonella* Heidelberg is an antibiotic resistant, pathogenic serovar belonging to this subspecies and has recently been implicated in a recent outbreak of Salmonellosis traced to ground turkey.

The current trend in poultry operations is to apply high levels of the Food Contact Substances (FCS) in a "finishing chiller" operation, where the FCS overflow water is captured into the main chiller water system, which conserves water and antimicrobial costs as well as supplement increased efficacy. Enviro Tech Chemical Services has recently applied for a Food Contact Notification to the FDA for use of high level FCS in finishing chiller applications. Therefore this study is designed to determine the efficacy of approximately 1,000 ppm peroxyacetic acid (PAA) from Perasan MP-2® and 350 ppm Br_2 from HB2 against *Salmonella* Heidelberg inoculated chicken halves at a short contact time. The level of planktonic *Salmonella* Heidelberg will also be measured.

Experiment Conditions

Part 1- *Salmonella* Heidelberg (Planktonic):

a) Control, 5 minutes: 100 ml inoculum in 20 L city water

b) PAA, 5 minutes: 100 ml inoculum in 20 L of 1100 ppm PAA(from Perasan MP-2)

c) Br_2, 5 minutes: 100 ml inoculum in 335 ppm (from activated HB2)

Part 2- *Salmonella* Heidelberg (Sessile):

a) Control, 5 minutes: Ten chicken halves- city water

b) PAA, 5 minutes: Ten chicken halves-1000 ppm (from Perasan MP-2)

c) Br_2, 5 minutes: Ten chicken halves-350 ppm (from activated HB2)

Note- Both Part 1 and Part 2 of this study were conducted at the same time, i.e., 10 of the *Salmonella* Heidelberg inoculated chicken halves were placed in each of the 20 L inoculated bins prior to treatment.

The Food Contact Substances

The MP-2 used to prepare the test solutions containing PAA was made from Perasan MP-2. Perasan MP-2 is a product that contains 15% peroxyacetic acid, 5.5% hydrogen peroxide, and 0.7% HEDP (hydroxyethylidene diphosphonic acid). The FCS has been issued prior approvals in the form of FCN's #887 and 908 for uses on meat and poultry.

HB-2 activation occurs by blending hydrogen bromide with a hypochlorite source. For this study, hypobromous acid was created on-site by combining hydrogen bromide (HB-2) and sodium hypochlorite directly to 20 L of city water containing the *Salmonella* Heidelberg and the 10 *Salmonella* Heidelberg inoculated chicken halves.

Methods

Test System:
Salmonella Heidelberg bacteria (ATCC 8326)

Salmonella Heidelberg bacteria were cultured in nutrient broth (Sigma, St. Louis, MS, lot number 095K0035) by incubation for two days at 35° C. The bacteria were separated from the nutrient broth by centrifugation, and carefully resuspended in approximately 800 mL of sterile phosphate buffer, which was later used to inoculate the test materials. The concentration of the *Salmonella* Heidelberg was measured in the inoculum by plating using 3M Petrifilm Enterobacteriaceae Plates which were then incubated at 35°C for 24 hours, upon which the plates were enumerated.

Fifteen whole, uncooked chickens were purchased from a local grocer. Each chicken was cut evenly into two halves after the removal of internal organs. This resulted in a total of 30 chicken halves.

100 mL of the *Salmonella* Heidelberg inoculum was added to each of three 30 gallon plastic storage bins containing 20 liters of chilled water.

The thirty chicken halves were patted dry with a towel, randomly split into three groups of ten, and sprayed with the remaining *Salmonella* inoculum, see Image 2.

All sets of ten chicken halves were placed in each of 30 gallon plastic storage bins containing 20 liters of the chilled water and *Salmonella* inoculum. Immediately thereafter, a calculated amount of each test antimicrobial was added to the appropriate bin. The actual concentration of PAA and Br_2 was measured using the Modified DPD method (see "Attachment A"). Separately, one bin containing 20 liters of the chilled water and *Salmonella* inoculum was designated as the control (no antimicrobial added) and also had a 5 minute contact time. All sets of control and challenge chicken halves

were agitated using gloved hands to simulate the movement through a typical finishing chiller during the 5 minute contact time.

After the challenge testing of *Salmonella,* at the time allotted, the plastic bin containing the PAA solution was treated with 90.6 g of erythorbic acid to neutralize any remaining PAA and H_2O_2 oxidant. Similarly, the Br_2 was neutralized in the bin containing the activated HB-2 by adding 10.4 g of erythorbic acid. After neutralization, a sample of the water was removed and plated for planktonic *Salmonella* using 3M Petrifilm Enterobacteriaceae Plates. The chicken halves were then removed and gently shaken three times to remove excess liquid and returned to a new, sterile plastic bag. 200 g of city water was introduced to each bag and subsequently tumbled gently for one minute to dislodge remaining sessile *Salmonella* bacteria. The water remaining at the bottom of the bag was plated in duplicate using 3M Petrifilm Enterobacteriaceae Plates. All plates were incubated at 35°C for 24 hours, upon which the plates were enumerated.

Results and Discussion

The *Salmonella* inoculum used to inoculate the 30 chicken halves yielded a *Salmonella* Heidelberg population of 3.09×10^8 or $\log_{10}$ 8.49.

The actual concentrations of the test solutions were measured by DPD method (see Attachment 'A') immediately after adding the test chemicals. The concentrations were 1102 ppm PAA and 335 ppm Br_2 from Perasan MP-2® and activated HB2, respectively.

Part 1- *Salmonella* Heidelberg (Planktonic)

Table 1 demonstrates the average number of planktonic *Salmonella* bacteria present in the test solutions containing the 10 chicken halves after the five minute contact time compared to the control. The $\log_{10}$ reduction in *Salmonella* Heidelberg compared to the control was >6.22 (>99.999%) for both the PAA and Br_2 test antimicrobials.

Table 1: Planktonic *Salmonella* bacteria 5 minute microbiological results

Description	Log10 CFU/ml Salmonella (average)	Log 10 Reduction	Percent Reduction
Control	6.22	N/A	N/A
PAA (MP-2)	0	>6.22	>99.9999%
Br2 (HB2)	0	>6.22	>99.9999%

Part 2- *Salmonella* Heidelberg (Sessile)

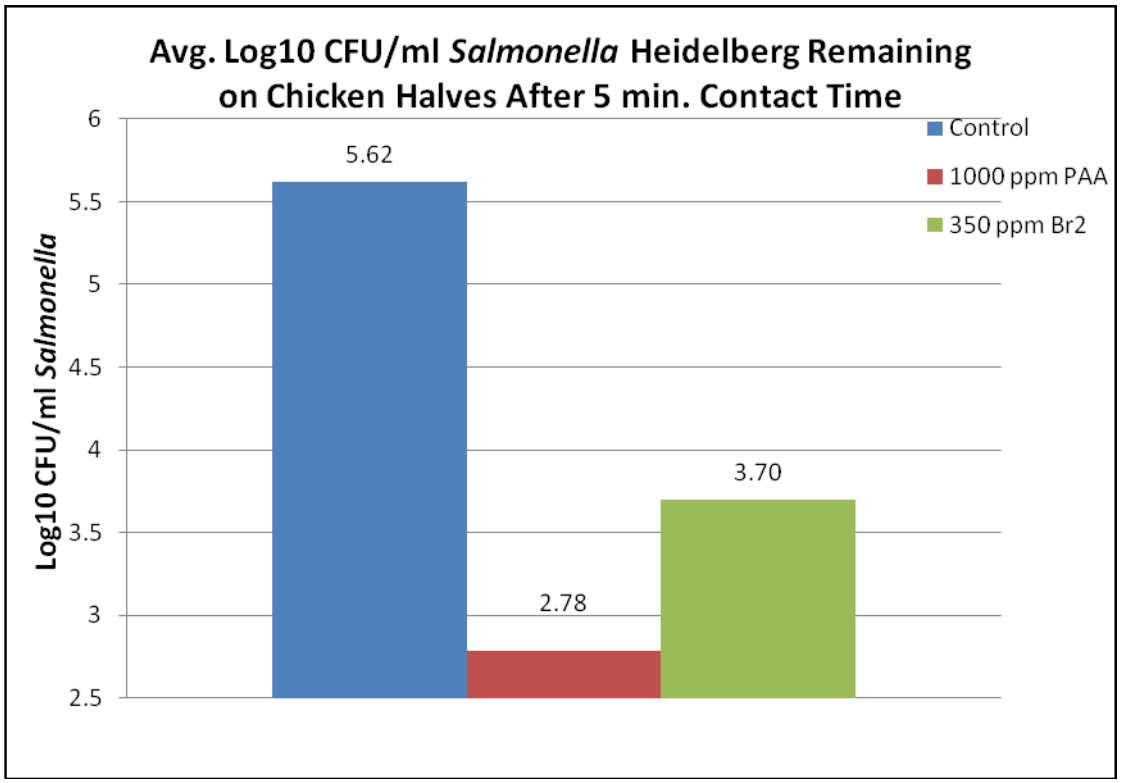
Table 2 and Figure 1 demonstrate the average number of bacteria left on the chicken after being submerged for five minutes in chilled city water (control) or PAA-challenge and

Br_2-challenge solutions. It can be seen that the control averaged a log_{10} of 5.62 CFU/ml. The log_{10} reduction in *Salmonella* Heidelberg bacteria on chicken halves submerged in a ~1000 ppm PAA solution compared to the control was 2.84 (99.855%). The log_{10} reduction in *Salmonella* Heidelberg bacteria on chicken halves submerged in a ~350 ppm Br_2 solution for 5 minutes, compared to the control, was 1.92 (98.798%).

Table 2: Sessile *Salmonella* bacteria microbiological results

Description	Log10 CFU/ml Salmonella (average)	Log 10 Reduction	Percent Reduction
Control	5.62	N/A	N/A
PAA (MP-2)	2.78	2.84	99.855
Br2 (HB2)	3.70	1.92	98.798

Figure 1:



Conclusions:

- There have been several successful studies performed by Enviro Tech Chemical Services which demonstrate the efficacy of several products against *Salmonella enterica*. Because of the recent outbreak of the antibiotic resistant *Salmonella* Heidelberg linked to ground turkey, it was decided to perform efficacy testing against this particular strain. This study was designed to give the reader a

reasonably accurate idea of the relative qualitative efficacy of peroxyacetic acid (from Perasan MP-2®) and activated HB-2 (hypobromous acid) against *Salmonella* Heidelberg bacteria in solution and on chicken when submerged for 5 minutes.

- The planktonic *Salmonella* Heidelberg present in solution was eradicated using 1102 ppm PAA and 335 ppm Br_2 after the five minute contact time compared to the control.

- There were log_{10} reductions of 2.84 (99.855%) and 1.92 (98.798%) by the use of PAA and activated HB-2, respectively. The Perasan MP-2 at 1100 ppm outperformed hypobromous acid at 335 ppm (reported as Br_2) because there was 6.9 times *more* PAA than bromine in terms of *molar* concentration (PAA = 76 gms/mole and Br_2 = 159.8 gms/mole). Therefore, on a mole per mole basis (ppm vs. ppm), hypobromous acid appears to be more efficacious than PAA on this organism. (0.258 log reduction for each 100 ppm of PAA vs. 0.582 log reduction for each 100 ppm of HB2).

- This study indicates that both PAA at approximately ~1100 ppm and activated HB2 (hypobromous acid) at approximately ~350 ppm are efficacious against *Salmonella* Heidelberg at a five minute contact time. However, there is a statistically relevant difference in the two chemistries. On a mole per mole basis (ppm vs. ppm), hypobromous acid appears to be more efficacious than PAA on this organism. (2.84 log ÷ 11 = **0.258** *log reduction* for each 100 ppm of PAA vs. 1.92 log ÷ 3.3 = **0.582** *log reduction* for each 100 ppm of HB2).

- No negative organoleptic observations were recorded for the bromine challenge. The PAA challenge bleached the chicken carcass during the 5 minute test. However, about 10-15 minutes after the conclusion of the PAA challenge test about half the normal yellow color of the carcass returned to normal. It is suggested that concentrations of the PAA approaching 1000 ppm be limited to shorter contact times than used herein to avoid bleaching of the poultry carcass.

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