The Efficacy of Peracetic Acid in Conjunction with Different Acid Blends Against *Salmonella* Heidelberg, *Campylobacter* Jejuni, and Aerobic Bacteria Inoculated Poultry

August 11, 2015

Enviro Tech Chemical Services, Inc.
Joseph Donabed B.S.

Background

A high level of concern and focus has developed over improving microbial efficacy during secondary processing, as this area of processing typically yields the highest positives for *Salmonella spp*. within the processing environment and has a substantial influence on the shelf life of the poultry as well. Peracetic acid is commonly used in sprays or dips prior to final packaging, but recently acidic-based processing aids have also been utilized. In particular, lactic acid and inorganic or organic acid blends have gained some popularity for this use.

BioFruit XF15 (15% PAA) and Perasan MP-2C (22% PAA) are FDA approved peracetic acid processing aids for use on meat, poultry, and seafood under FCNs 699, 887, 908, 1132, and 1419. Peracetic acid has a pKa of 8.20 at 25°C[1]. Therefore the lower the pH the greater the concentration of active PAA in solution, which in theory should increase the overall efficacy. Using this theory, a lactic acid, hydrochloric acid, and phosphoric acid blended product was developed for use in Part 1 of this report. The purpose of Part 2 was to determine whether the lactic acid blend in conjunction with peracetic acid improved microbial efficacy. In addition, Part 2 of the study investigated whether a lactic acid blend can increase the shelf-life of retail poultry products, or whether the increased acidity is the key factor and not the *type or blend* of acid used.

It has been suggested that lactic acid imparts some type of longer term bacteriostatic or bactericidal effect on the surface of the poultry product. The purpose of this detailed report will be to (1) evaluate the differences, if any, using 2 types of PAA (15% and 22% PAA) against Salmonella and APC (Aerobic Plate Counts) over a 25 day refrigerated evaluation period ; (2) Evaluate a PAA solution (200 and 400 ppm PAA) alone <u>and</u> in a pH adjusted solution using a lactic acid-based acid formulation to a pH of 1.8 and 2.8 over a 25 day refrigerated evaluation period; (3) Evaluate 200 and 400 ppm of PAA alone <u>and</u> in conjunction with two other *non-lactic* acid formulations at pH's of 1.5 and 1.8 against Campylobacter jejuni and APC over a 25 day refrigerated evaluation period.

[1] Dean, J.A. (ed.). Lange's Handbook of Chemistry. 13 ed. New York, NY: McGraw-Hill Book Co., 1985., p. 5-53

Part 1

This section evaluates the effect and/or differences of two independent PAA formulations: BioFruit XF15 (15% PAA) and Perasan MP-2C (22% PAA). In addition, this section will determine the overall influence of a lactic acid-based formulation alone and in combination with the PAA products at two pH points (pH 1.8 and 2.8) and at two ppm concentrations (200 and 400 ppm). The primary organism challenge was *Salmonella* Heidelberg. However, on day 20 and 25, APC was also added to the test regime, along with an odor evaluation score. The odor emanating from the stored poultry was on a 1-5 scale, with 5 being a rancid odor. It should be noted that the two types of PAA (15% and 22%) in this study performed statistically the same, so the average readings in terms of efficacy and observations are reported in the following tables. On day 20 and 25, the PAA (both concentrations) at an acid adjusted pH 1.8 clearly outperformed all other test conditions, so it was decided to use Campylobacter and APC for Part 2 of this challenge study.

Part 2

From Part 1 it was determined that the acid formula alone performed moderately at best until the later stages of the study at day 20 and 25. Therefore, in Part 2, it was decided to utilize two other alternate non-lactic acid formulations in an attempt to determine whether lactic acid was the contributing component, or whether it was the lower acidity itself on the surface of the poultry that showed bacterial inhibition (bacteriostatic) effects. In addition, all challenge tests in Part 1 (at pH 2.8) did not perform well, so it was decided to use pH 1.5 and 1.8 as the pH points for this study.

Part 1: Efficacy against *Salmonella* Heidelberg and Aerobic Bacteria

Materials and Methods

BioFruit XF15 (Lot# 825-020915-1) was analyzed by iodometric titration and yielded a peracetic acid concentration of 15.55% and hydrogen peroxide concentration of 5.86%. Perasan MP-2C (Lot# 844-012915-1) was also analyzed and had a peracetic acid concentration of 21.95% and a hydrogen peroxide concentration of 5.18%.

The lactic acid blend used in Part 1 consisted of hydrochloric acid, lactic acid, and phosphoric acid.

Table 1 lists the solutions that were tested against *Salmonella* Heidelberg inoculated poultry and the dose for each product in 5-gallons of water

Solution Description	Dose BioFruit XF15 (mL)	Dose Perasan MP-2C (mL)	Lactic acid blend (mL)
Control (Water Only)	0	0	0
200 ppm PAA BioFruit XF15	22.00	0	0
400 ppm PAA BioFruit XF15	44.00	0	0
200 ppm PAA Perasan MP-2C	0	15.50	0
400 ppm PAA Perasan MP-2C	0	31.00	0
Lactic Acid Blend to pH 1.8	0	0	150
Lactic Acid Blend to pH 2.8	0	0	90
200 ppm PAA BF XF15 at pH 1.8	22.00	0	150

200 ppm PAA MP-2 at pH 2.8	22.00	0	90
400 ppm PAA MP-2 at pH 1.8	44.00	0	150
400 ppm PAA MP-2 at pH 2.8	44.00	0	90
200 ppm PAA MP-2C at pH 1.8	0	15.50	150
200 ppm PAA MP-2C at pH 2.8	0	15.50	90
400 ppm PAA MP-2C at pH 1.8	0	31.00	150
400 ppm PAA MP-2C at pH 2.8	0	31.00	90

Preparation of *Salmonella* Heidelberg Culture

A freeze-dried pellet of *Salmonella* Heidelberg was reconstituted in 10 mL of Brain Heart Infusion Broth (Criterion Cat. No. C5140) and vortexed for 30 seconds. Next, 1 mL aliquots were taken and plated on Tryptic Soy Agar with 5% Sheep's Blood (Hardy Diagnostics Cat no. A600). This technique was repeated three times yielding a total of 30 culture plates. The plates were incubated at 37°C for 48 hours. After the 48 hour incubation period, the bacterial colonies were transferred to 30 L of reverse osmosis water using and L-shaped spreader. The bacterial solution was homogenized. A total of 315 chicken legs (drum sticks) were purchased from the local grocery. The chicken legs were submerged in the 30 L bacterial culture for 5 minutes, removed and drained of excess liquid, then allowed to dry for 30 minutes to ensure bacterial attachment.

Treatment with Antimicrobial Solutions

After the chicken legs were dried, 21 of the 315 chicken legs were submerged into the respective solutions listed in **Table 1** for 15 seconds. After the 15 second treatment time, the chicken legs were allowed to drain for 2 minutes then 3 of the 18 treated chicken legs were placed into individual sterile stomacher bags and the remaining 18 legs were transferred to a single sterile stomacher bag and labeled. These bags of chicken legs were refrigerated at 2.2°C for future testing. A total of 50 mL of sterile D/E Neutralizing Broth (Criterion Cat No.: C7371) was added to the individual stomacher bags containing three chicken legs per bag to neutralized any remaining antimicrobial and then the legs were vigorously agitated for 60 seconds. Aliquots were taken from each bag, serially diluted, and plated on 3M Enterobacteriaceae Petrifilms™. Petrifilms™ were incubated at 35°C for 24 hours then enumerated. On day 20, 24 and 25 after treatment, aerobic plate counts were taken by plating the D/E Neutralizing broth wash on 3M Aerobic Plate Count (APC) Petrifilms™. The APC Petrifilms™ were incubated at 35°C for 48 hours then enumerated. 5, 10, 15, 20, and 25 days after treatment, 3 chicken legs were removed for each of the sealed refrigerated bags and testing was repeated.

Results and Discussion

Table 2 lists the concentrations and pH of the solutions used to treat the chicken legs

Description	PAA Concentration (ppm)	pH
Water Only	NA	7.62
200 ppm PAA BioFruit XF15	207.6	4.30
200 ppm PAA Perasan MP-2C	204.7	4.31
400 ppm PAA BioFruit XF15	404.5	3.98
400 ppm PAA Perasan MP-2C	395.9	3.94
Lactic acid blend to pH 1.8	NA	1.82
Lactic acid blend to pH 2.8	NA	2.79
200 ppm PAA BF XF15 at pH 1.8	208.7	1.87
200 ppm PAA BF XF15 at pH 2.8	203.3	2.84
400 ppm PAA BF XF15 at pH 1.8	408.7	1.84
400 ppm PAA BF XF15 at pH 2.8	413.0	2.85
200 ppm PAA MP-22 at pH 1.8	199.0	2.81
200 ppm PAA MP-22 at pH 2.8	194.7	2.83
400 ppm PAA MP-22 at pH 1.8	400.2	1.79
400 ppm PAA MP-22 at pH 2.8	410.9	2.86

The efficacy of the BioFruit XF15 and Perasan MP-2C were deemed to not be statically different. Therefore the following efficacy results were reported as averages based on PAA concentration.

Table 3 details the average reduction $\log_{10}$ (CFU/mL) *Salmonella* Heidelberg over a 25 day time interval and aerobic bacteria from day 20-25.

***Salmonella* Heidelberg** **Day 0**

Description	Average $\log_{10}$	Average $\log_{10}$ Reduction	% Reduction
Water Only	3.60	NA	NA
200 ppm PAA	2.58	1.02	90.45
400 ppm PAA	2.17	1.43	96.28
Lacticide pH 1.8	3.37	0.23	41.12
Lacticide pH 2.8	3.54	0.06	12.90
200 ppm PAA/pH 1.8	2.13	1.47	96.61
200 ppm PAA/pH 2.8	2.36	1.24	94.25
400 ppm PAA/pH 1.8	1.84	1.76	98.26
400 ppm PAA/pH 2.8	2.06	1.54	97.12

Salmonella Heidelberg **Day 5**

Description	Average log_{10}	Average log_{10} Reduction	% Reduction
Water Only	2.87	NA	NA
200 ppm PAA	2.38	0.49	67.64
400 ppm PAA	2.22	0.65	77.61
Lacticide pH 1.8	2.52	0.35	55.33
Lacticide pH 2.8	2.79	0.08	16.82
200 ppm PAA/pH 1.8	1.84	1.03	90.67
200 ppm PAA/pH 2.8	2.33	0.54	71.16
400 ppm PAA/pH 1.8	1.72	1.15	92.92
400 ppm PAA/pH 2.8	2.19	0.68	79.11

Salmonella Heidelberg **Day 10**

Description	Average log_{10}	Average log_{10} Reduction	% Reduction
Water Only	2.95	NA	NA
200 ppm PAA	2.29	0.66	78.12
400 ppm PAA	2.18	0.77	83.02
Lacticide pH 1.8	2.53	0.42	61.98
Lacticide pH 2.8	2.82	0.13	25.87
200 ppm PAA/pH 1.8	1.86	1.09	91.87
200 ppm PAA/pH 2.8	2.39	0.56	72.46
400 ppm PAA/pH 1.8	1.75	1.20	93.69
400 ppm PAA/pH 2.8	2.23	0.72	80.95

Salmonella Heidelberg **Day 15**

Description	Average log_{10}	Average log_{10} Reduction	% Reduction
Water Only	3.03	NA	NA
200 ppm PAA	2.48	0.55	72.08
400 ppm PAA	2.41	0.62	76.01
Lacticide pH 1.8	2.79	0.24	42.35
Lacticide pH 2.8	2.91	0.12	23.53
200 ppm PAA/pH 1.8	2.36	0.67	78.54
200 ppm PAA/pH 2.8	2.44	0.59	74.48
400 ppm PAA/pH 1.8	2.22	0.81	84.59
400 ppm PAA/pH 2.8	2.34	0.69	79.77

***Salmonella* Heidelberg** **Day 20**

Description	Average $\log_{10}$	Average $\log_{10}$ Reduction	% Reduction	Odor Score*
Water Only	3.63	NA	NA	3
200 ppm PAA	3.19	0.43	62.99	1.5
400 ppm PAA	3.14	0.48	66.98	1.5
Lacticide pH 1.8	2.90	0.73	81.20	1
Lacticide pH 2.8	3.52	0.10	20.96	2.5
200 ppm PAA/pH 1.8	2.58	1.04	90.98	1
200 ppm PAA/pH 2.8	2.86	0.76	82.69	2
400 ppm PAA/pH 1.8	2.38	1.25	94.33	1
400 ppm PAA/pH 2.8	3.02	0.61	75.30	1.5

***Salmonella* Heidelberg** **Day 25**

Description	Average $\log_{10}$	Average $\log_{10}$ Reduction	% Reduction	Odor Score*
Water Only	4.89	NA	NA	5
200 ppm PAA	4.18	0.70	80.17	3.5
400 ppm PAA	4.01	0.88	86.68	3.5
Lacticide pH 1.8	4.21	0.67	78.69	2
Lacticide pH 2.8	4.40	0.49	67.50	4.5
200 ppm PAA/pH 1.8	2.80	2.08	99.17	2
200 ppm PAA/pH 2.8	4.17	0.72	80.75	4
400 ppm PAA/pH 1.8	2.52	2.37	99.57	2
400 ppm PAA/pH 2.8	4.05	0.84	85.41	3.5

Aerobic Plate (APC) **Day 20**

Description	Average $\log_{10}$	Average $\log_{10}$ Reduction	% Reduction	Odor Score*
Water Only	4.95	NA	NA	3
200 ppm PAA	4.43	0.52	69.87	1.5
400 ppm PAA	4.03	0.92	87.98	1.5
Lacticide pH 1.8	4.68	0.27	46.44	1
Lacticide pH 2.8	4.89	0.06	12.40	2.5
200 ppm PAA/pH 1.8	2.77	2.18	99.34	1
200 ppm PAA/pH 2.8	4.14	0.81	84.51	2
400 ppm PAA/pH 1.8	2.25	2.70	99.80	1
400 ppm PAA/pH 2.8	3.97	0.98	89.51	1.5

Aerobic Plate (APC) **Day 25**

Description	Average log_{10}	Average log_{10} Reduction	% Reduction	Odor Score*
Water Only	8.22	NA	NA	5
200 ppm PAA	5.45	2.78	99.83	3.5
400 ppm PAA	5.34	2.88	99.87	3.5
Lacticide pH 1.8	6.64	1.58	97.39	2
Lacticide pH 2.8	7.22	1.00	90.06	4.5
200 ppm PAA/pH 1.8	4.32	3.90	99.99	2
200 ppm PAA/pH 2.8	5.41	2.81	99.85	4
400 ppm PAA/pH 1.8	4.08	4.15	99.99	2
400 ppm PAA/pH 2.8	5.41	2.82	99.85	3.5

*Odor Score: 1 is the least odor and 5 is the worst odor.

Discussion

- On day 20, and especially on day 25, it became clear that although the lactic acid blend (Lacticide) held down odor well, it did not perform as well against 200 ppm PAA or 400 ppm PAA at pH 1.8 or 2.8. For *S.* Heidelberg, PAA at 200 and 400 ppm showed log_{10} reduction of 1.04 and 1.25 (CFU/mL) vs. lactic acid at 0.73 CFU/mL. For APC, 200 and 400 ppm PAA showed an average log_{10} reduction of 3.9 and 4.15, vs. lactic acid at 1.58 CFU/mL.
- All of the PAA challenge tests performed substantially better at pH 1.8 vs. pH 2.8, which indicates there is a marked improvement of pH adjusted PAA performance over time compared to unadjusted PAA or lactic acid used alone.
- Although the lactic acid blend did not perform as well in terms of efficacy as pH adjusted PAA, it is clear that it had an observable effect on reducing odor, similar to the PAA at pH 1.8.

Part 2: Efficacy against *Campylobacter jejuni* and Aerobic Bacteria

The second part of this experiment was designed to determine whether the efficacy results achieved in **Part 1** were due to the specific lactic acid blend or simply due to the pH depression. Also, it will be important to determine whether decreasing the pH to 1.5 increases overall efficacy and increases product shelf-life.

Treatment with Antimicrobial Solutions

Two different acids blends were chosen for this study: Citric/hydrochloric acid blend and a citric/sulfuric/phosphoric acid blend.

Table 4 lists the solutions that were tested against *Campylobacter jejuni* inoculated poultry and the dose for each product in 5-gallons of water

Solution Description	Dose BioFruit XF15 (mL)	Dose Acid blend (mL)
Control (Water)	0	0
200 ppm PAA Only	22	0
400 ppm PAA Only	44	0
200 ppm PAA pH 1.5 HCl/Citric	22	56
200 ppm PAA pH 1.8 HCl/Citric	22	50
200 ppm PAA pH 1.5 citric/sulfuric/phos.	22	88
200 ppm PAA pH 1.8 citric/sulfuric/phos.	22	77
400 ppm PAA pH 1.5 HCl/Citric	44	56
400 ppm PAA pH 1.8 HCl/Citric	44	50
400 ppm PAA pH 1.5 citric/sulfuric/phos.	44	88
400 ppm PAA pH 1.8 citric/sulfuric/phos.	44	77

Preparation of *Campylobacter jejuni* Culture

Campylobacter jejuni (ATCC 33291) freeze dried pellet was cultured in Bolton Broth (Sigma Aldrich, lot number BCBB7257) containing 5% defibrinated sheep blood (Hardy Diagnostics) by anaerobic incubation at 42.3° C for 48 hours. The bacteria were separated from the nutrient broth by centrifugation. One mL of the concentrated bacteria mixture was removed and plated on Campy Cefex Agar (Hardy Diagnostics). This was repeated ten times to achieve a total of ten Campylobacter-inoculated Campy Cefex Agar plates. The plates were kept under anaerobic atmosphere and incubated for 48 hours at 42.3°C. After the 48 hour incubation period, the bacterial colonies were transferred to 30 L of reverse osmosis water using and L-shaped spreader. The bacterial solution was homogenized. A total of 200 chicken legs (drum sticks) were purchased from the local grocery. The chicken legs were submerged in the 30 L bacterial culture for 5 minutes, removed and drained of excess liquid, then allowed to dry for 30 minutes to ensure bacterial attachment.

Treatment with Antimicrobial Solutions

After the chicken legs were dried, 20 of the 200 drum sticks were submerged into the respective solutions listed in **Table 4** for 15 seconds. After the 15 second treatment time, the chicken legs were allowed to drain for 2 minutes then 3 of the 20 treated chicken legs were placed into individual sterile stomacher bags and the remaining 17 legs were transferred to a single sterile stomacher bag and labeled. These bags of chicken legs were sealed and refrigerated at 2.2°C for future testing. A total of 50 mL of sterile D/E Neutralizing Broth (Criterion Cat No.: C7371) was added to the individual stomacher bags containing three chicken legs per bag to neutralized any remaining antimicrobial and then the legs were vigorously agitated for 60 seconds. Aliquots were taken from each bag, serially diluted, and plated for *C. jejuni* and APC on Campy Cefex Agar plates and 3M Aerobic Plate Count (APC) Petrifilms™. Agar plates were incubated at 42°C for48 hours then enumerated and Petrifilms™ were incubated at 33°F for 48 hours then enumerated. 5, 10, 15, 20, and 25 days after treatment, three drumsticks were removed from each of the sealed refrigerated bags and testing was repeated.

Results

Campylobacter jejuni Testing

Day 0	*C. Jejuni*		
Description	**Avg. $\log_{10}$ (CFU/mL)**	**Avg. $\log_{10}$ Reduction (CFU/mL)**	**% Reduction**
Control (Water)	4.55	NA	NA
200 ppm PAA Only	3.45	1.10	92.13
400 ppm PAA Only	3.26	1.30	94.94
200 ppm PAA pH 1.5 HCl/Citric	2.61	1.94	98.86
200 ppm PAA pH 1.8 HCl/Citric	**2.68**	**1.88**	**98.67**
200 ppm PAA pH 1.5 citric/sulfuric/phos	2.60	1.95	98.89
200 ppm PAA pH 1.8 citric/sulfuric/phos	**2.64**	**1.91**	**98.78**
400 ppm PAA pH 1.5 HCl/Citric	2.02	2.53	99.71
400 ppm PAA pH 1.8 HCl/Citric	**2.13**	**2.42**	**99.62**
400 ppm PAA pH 1.5 citric/sulfuric/phos	2.13	2.42	99.62
400 ppm PAA pH 1.8 citric/sulfuric/phos	**2.25**	**2.31**	**99.51**

Day 5	*C. Jejuni*		
Description	**Avg. log_{10} (CFU/mL)**	**Avg. log_{10} Reduction (CFU/mL)**	**% Reduction**
Control (Water)	4.44	NA	NA
200 ppm PAA Only	2.18	2.27	99.46
400 ppm PAA Only	1.98	2.47	99.66
200 ppm PAA pH 1.5 HCl/Citric	1.75	2.70	99.80
200 ppm PAA pH 1.8 HCl/Citric	**1.81**	**2.63**	**99.77**
200 ppm PAA pH 1.5 citric/sulfuric/phos	1.79	2.66	99.78
200 ppm PAA pH 1.8 citric/sulfuric/phos	**1.83**	**2.62**	**99.76**
400 ppm PAA pH 1.5 HCl/Citric	1.38	3.06	99.91
400 ppm PAA pH 1.8 HCl/Citric	**1.49**	**2.95**	**99.89**
400 ppm PAA pH 1.5 citric/sulfuric/phos	1.45	3.00	99.90
400 ppm PAA pH 1.8 citric/sulfuric/phos	**1.52**	**2.93**	**99.88**

Day 10	*C. Jejuni*		
Description	**Avg. log_{10} (CFU/mL)**	**Avg. log_{10} Reduction (CFU/mL)**	**% Reduction**
Control (Water)	4.13	NA	NA
200 ppm PAA Only	2.00	2.13	99.25
400 ppm PAA Only	1.82	2.31	99.51
200 ppm PAA pH 1.5 HCl/Citric	1.62	2.50	99.69
200 ppm PAA pH 1.8 HCl/Citric	**1.74**	**2.39**	**99.59**
200 ppm PAA pH 1.5 citric/sulfuric/phos	1.65	2.47	99.66
200 ppm PAA pH 1.8 citric/sulfuric/phos	**1.74**	**2.39**	**99.59**
400 ppm PAA pH 1.5 HCl/Citric	1.28	2.85	99.86
400 ppm PAA pH 1.8 HCl/Citric	**1.32**	**2.81**	**99.84**
400 ppm PAA pH 1.5 citric/sulfuric/phos	1.34	2.79	99.84
400 ppm PAA pH 1.8 citric/sulfuric/phos	**1.40**	**2.73**	**99.81**

Day 15	***C. Jejuni***		
Description	**Avg. log_{10} (CFU/mL)**	**Avg. log_{10} Reduction (CFU/mL)**	**% Reduction**
Control (Water)	4.02	NA	NA
200 ppm PAA Only	1.96	2.06	99.12
400 ppm PAA Only	1.71	2.31	99.51
200 ppm PAA pH 1.5 HCl/Citric	1.52	2.50	99.69
200 ppm PAA pH 1.8 HCl/Citric	**1.62**	**2.40**	**99.60**
200 ppm PAA pH 1.5 citric/sulfuric/phos	1.48	2.54	99.71
200 ppm PAA pH 1.8 citric/sulfuric/phos	**1.61**	**2.41**	**99.61**
400 ppm PAA pH 1.5 HCl/Citric	0.70	3.32	99.95
400 ppm PAA pH 1.8 HCl/Citric	**1.18**	**2.84**	**99.86**
400 ppm PAA pH 1.5 citric/sulfuric/phos	0.85	3.18	99.93
400 ppm PAA pH 1.8 citric/sulfuric/phos	**1.08**	**2.94**	**99.89**

Day 20	***C. Jejuni***			
Description	**Avg. log_{10} (CFU/mL)**	**Avg. log_{10} Reduction (CFU/mL)**	**% Reduction**	**Odor Score**
Control (Water)	3.88	NA	NA	3.5
200 ppm PAA Only	1.65	2.23	99.41	1.5
400 ppm PAA Only	1.51	2.37	99.57	1.5
200 ppm PAA pH 1.5 HCl/Citric	1.20	2.67	99.79	1
200 ppm PAA pH 1.8 HCl/Citric	**1.34**	**2.53**	**99.71**	**1**
200 ppm PAA pH 1.5 citric/sulfuric/phos	1.26	2.61	99.76	1
200 ppm PAA pH 1.8 citric/sulfuric/phos	**1.29**	**2.59**	**99.74**	**1**
400 ppm PAA pH 1.5 HCl/Citric	0.00	3.88	99.9	1
400 ppm PAA pH 1.8 HCl/Citric	**0.78**	**3.10**	**99.92**	**1**
400 ppm PAA pH 1.5 citric/sulfuric/phos	0.00	3.88	99.9	1
400 ppm PAA pH 1.8 citric/sulfuric/phos	**0.60**	**3.27**	**99.95**	**1**

Day 25	*C. Jejuni*			
Description	**Avg. $\log_{10}$ (CFU/mL)**	**Avg. $\log_{10}$ Reduction (CFU/mL)**	**% Reduction**	**Odor Score**
Control (Water)	3.81	NA	NA	5
200 ppm PAA Only	1.32	2.48	99.67	2
400 ppm PAA Only	1.28	2.53	99.70	2
200 ppm PAA pH 1.5 HCl/Citric	0.90	2.90	99.88	1.5
200 ppm PAA pH 1.8 HCl/Citric	**1.08**	**2.73**	**99.81**	**1.5**
200 ppm PAA pH 1.5 citric/sulfuric/phos	1.04	2.76	99.83	1.5
200 ppm PAA pH 1.8 citric/sulfuric/phos	**1.18**	**2.63**	**99.77**	**1.5**
400 ppm PAA pH 1.5 HCl/Citric	0.00	3.81	99.9	1
400 ppm PAA pH 1.8 HCl/Citric	**0.00**	**3.81**	**99.9**	**1.5**
400 ppm PAA pH 1.5 citric/sulfuric/phos	0.00	3.81	99.9	1
400 ppm PAA pH 1.8 citric/sulfuric/phos	**0.00**	**3.81**	**99.9**	**1.5**

APC Testing

Day 0	APC		
Description	**Avg. $\log_{10}$ (CFU/mL)**	**Avg. $\log_{10}$ Reduction (CFU/mL)**	**% Reduction**
Control (Water)	6.55	NA	NA
200 ppm PAA Only	5.93	0.62	76.25
400 ppm PAA Only	5.54	1.01	90.22
200 ppm PAA pH 1.5 HCl/Citric	5.72	0.84	85.47
200 ppm PAA pH 1.8 HCl/Citric	**5.79**	**0.76**	**82.68**
200 ppm PAA pH 1.5 citric/sulfuric/phos	5.76	0.79	83.79
200 ppm PAA pH 1.8 citric/sulfuric/phos	**5.83**	**0.72**	**81.00**
400 ppm PAA pH 1.5 HCl/Citric	5.32	1.23	94.13
400 ppm PAA pH 1.8 HCl/Citric	**5.51**	**1.05**	**91.06**
400 ppm PAA pH 1.5 citric/sulfuric/phos	5.30	1.25	94.41
400 ppm PAA pH 1.8 citric/sulfuric/phos	**5.52**	**1.04**	**90.78**

Day 5	APC		
Description	**Avg. log_{10} (CFU/mL)**	**Avg. log_{10} Reduction (CFU/mL)**	**% Reduction**
Control (Water)	6.29	NA	NA
200 ppm PAA Only	5.52	0.78	83.25
400 ppm PAA Only	5.15	1.15	92.89
200 ppm PAA pH 1.5 HCl/Citric	5.26	1.04	90.86
200 ppm PAA pH 1.8 HCl/Citric	**5.38**	**0.91**	**87.71**
200 ppm PAA pH 1.5 citric/sulfuric/phos	5.18	1.12	92.38
200 ppm PAA pH 1.8 citric/sulfuric/phos	**5.37**	**0.92**	**88.07**
400 ppm PAA pH 1.5 HCl/Citric	4.96	1.33	95.32
400 ppm PAA pH 1.8 HCl/Citric	**5.04**	**1.25**	**94.44**
400 ppm PAA pH 1.5 citric/sulfuric/phos	4.98	1.32	95.19
400 ppm PAA pH 1.8 citric/sulfuric/phos	**5.05**	**1.25**	**94.33**

Day 10	APC		
Description	**Avg. log_{10} (CFU/mL)**	**Avg. log_{10} Reduction (CFU/mL)**	**% Reduction**
Control (Water)	6.57	NA	NA
200 ppm PAA Only	5.62	0.95	88.78
400 ppm PAA Only	5.08	1.49	96.79
200 ppm PAA pH 1.5 HCl/Citric	5.18	1.40	95.99
200 ppm PAA pH 1.8 HCl/Citric	**5.29**	**1.28**	**94.76**
200 ppm PAA pH 1.5 citric/sulfuric/phos	5.08	1.49	96.79
200 ppm PAA pH 1.8 citric/sulfuric/phos	**5.33**	**1.25**	**94.33**
400 ppm PAA pH 1.5 HCl/Citric	4.90	1.68	97.89
400 ppm PAA pH 1.8 HCl/Citric	**4.93**	**1.64**	**97.71**
400 ppm PAA pH 1.5 citric/sulfuric/phos	4.88	1.69	97.97
400 ppm PAA pH 1.8 citric/sulfuric/phos	**4.95**	**1.62**	**97.59**

Day 15	**APC**		
Description	**Avg. log_{10} (CFU/mL)**	**Avg. log_{10} Reduction (CFU/mL)**	**% Reduction**
Control (Water)	6.73	NA	NA
200 ppm PAA Only	5.79	0.94	88.57
400 ppm PAA Only	5.38	1.35	95.50
200 ppm PAA pH 1.5 HCl/Citric	5.41	1.31	95.13
200 ppm PAA pH 1.8 HCl/Citric	**5.51**	**1.22**	**94.01**
200 ppm PAA pH 1.5 citric/sulfuric/phos	5.46	1.27	94.59
200 ppm PAA pH 1.8 citric/sulfuric/phos	**5.49**	**1.24**	**94.21**
400 ppm PAA pH 1.5 HCl/Citric	4.98	1.75	98.22
400 ppm PAA pH 1.8 HCl/Citric	**5.07**	**1.66**	**97.80**
400 ppm PAA pH 1.5 citric/sulfuric/phos	5.02	1.71	98.06
400 ppm PAA pH 1.8 citric/sulfuric/phos	**5.12**	**1.61**	**97.54**

Day 20	**APC**			
Description	**Avg. log_{10} (CFU/mL)**	**Avg. log_{10} Reduction (CFU/mL)**	**% Reduction**	**Odor Score**
Control (Water)	6.94	NA	NA	3.5
200 ppm PAA Only	6.05	0.88	86.89	1.5
400 ppm PAA Only	5.83	1.11	92.22	1.5
200 ppm PAA pH 1.5 HCl/Citric	5.88	1.06	91.30	1
200 ppm PAA pH 1.8 HCl/Citric	**5.94**	**1.00**	**89.92**	**1**
200 ppm PAA pH 1.5 citric/sulfuric/phos	5.33	1.61	97.54	1
200 ppm PAA pH 1.8 citric/sulfuric/phos	**5.96**	**0.97**	**89.39**	**1**
400 ppm PAA pH 1.5 HCl/Citric	5.06	1.88	98.68	1
400 ppm PAA pH 1.8 HCl/Citric	**5.23**	**1.71**	**98.05**	**1**
400 ppm PAA pH 1.5 citric/sulfuric/phos	5.05	1.89	98.70	1
400 ppm PAA pH 1.8 citric/sulfuric/phos	**5.27**	**1.67**	**97.86**	**1**

Day 25	APC			
Description	**Avg. log_{10} (CFU/mL)**	**Avg. log_{10} Reduction (CFU/mL)**	**% Reduction**	**Odor Score**
Control (Water)	7.07	NA	NA	5
200 ppm PAA Only	6.12	0.95	88.74	2
400 ppm PAA Only	5.86	1.21	93.86	2
200 ppm PAA pH 1.5 HCl/Citric	5.74	1.33	95.31	1.5
200 ppm PAA pH 1.8 HCl/Citric	**5.81**	**1.26**	**94.46**	**1.5**
200 ppm PAA pH 1.5 citric/sulfuric/phos	5.77	1.30	94.97	1.5
200 ppm PAA pH 1.8 citric/sulfuric/phos	**5.81**	**1.26**	**94.46**	**1.5**
400 ppm PAA pH 1.5 HCl/Citric	4.91	2.16	99.30	1
400 ppm PAA pH 1.8 HCl/Citric	**5.05**	**2.02**	**99.04**	**1.5**
400 ppm PAA pH 1.5 citric/sulfuric/phos	4.95	2.12	99.24	1
400 ppm PAA pH 1.8 citric/sulfuric/phos	**5.11**	**1.96**	**98.91**	**1.5**

Discussion

- It is evident that the Campylobacter diminished in prevalence as time progressed. The control on day 1 contained an average log_{10} of 4.55 CFU/mL, whereas after 25 days it was only 3.81 CFU/mL.
- For *C. jejuni* at day 25, 200 ppm PAA used alone or acid adjusted to pH 1.5 or 1.8 performed about the same, with the exception of 200 ppm using the HCl/citric acid blend, which had slightly better efficacy than the citric/sulfuric/phosphoric acid blend and the PAA alone. However, all of the 200 ppm PAA results at day 25 were statistically non-distinguishable.
- As expected, the control population of Aerobic Plate Counts (APC) rose on average from a log_{10} of 6.55 CFU/mL on day 1 up to an average log_{10} of 7.07 CFU/mL on day 25.
- For APC and odor, PAA used alone did not perform as well as the other pH adjusted formulations on day 25.
- A moderate difference was noted on day 25 with the pH adjusted 400 ppm PAA formulation at pH 1.5 vs. pH 1.8, with pH 1.5 performing somewhat better than the challenge at pH 1.8 (a log_{10} difference of 0.15 CFU/mL).
- 200 ppm PAA either with or without additional acid performed similarly against APC organisms and on the odor score.

Conclusions

- When comparing the day 25 APC counts of Part 1 vs. Part 2, one must note that the control in Part 1 contained an average log_{10} of 8.22 CFU/mL, but the control on day 25 in Part 2 contained only 7.07 CFU/mL, which is a log_{10} difference of 1.15 CFU/mL. Thus, the pH adjusted PAA at 400 ppm performed the best in both Part 1 and Part 2.
- In terms of cost, if we assume all the 3 types of acids used in this study have equivalent costs per gallon, the two acid blends used in Part 2 are far more cost effective than the lactic acid used in Part 1. For example, all tests were done using 5 gal volumes. Part 1 (Table 1) utilized acid at the rate of 30 ml per gallon of water to achieve a pH of 1.8. Similarly, the two acids used in Part 2 (Table 4) required 11 mL and 15 mL, respectively, per gallon of water to achieve a pH of 1.8.
- In terms of efficacy and odor score, 400 ppm PAA adjusted to a lower pH performed better in all tests performed in this study. 200 ppm PAA adjusted to lower pH's also performed better than unadjusted PAA or the acid alone that was used in Part 1.
- In Part 1 it became clear that adjusting pH from 1.8 to 2.8 produced less favorable results. On the other hand, in Part 2 one can see that pH 1.5 performed only slightly better than pH 1.8 when used to adjust PAA solutions. It may be debatable if the lower acid feed rate to achieve pH 1.5 may be worth the cost, but it is very evident that allowing pH to rise above 1.8 (Part 1) would likely have less favorable results.
- It does not appear that lactic acid formulations exhibit synergism any more or less than conventional acids tested herein, when used in conjunction with PAA solutions. It appears the effect of the acids is a lowering of pH on the poultry surface, which results in an inhibitory bacteriostatic effect. Therefore, the cost of these more exotic acid product offerings may not be justified.
- Acid pH adjusted PAA used in secondary processing (at pH range of 1.5-1.8) will perform measurably better than acid products or PAA used alone.
- In Part 2, 400 ppm pH adjusted PAA almost completely eliminated *Campylobacter jejuni* after the 25 day trial and also had a very good efficacy effect on Salmonella as well.
- 400 ppm of a 22% PAA concentrate, such as Perasan MP-2C, requires only 0.21 mL per gallon of process water, so it is 10-13 times more cost effective to raise the PAA concentration in the solution compared to adjusting the acid component to achieve a lower pH (acids cost less per gallon but one needs to add 11-15 mL per gal to achieve a pH of 1.8).
- PH adjusting a PAA solution at 400 ppm as PAA to a pH of about 1.8 has demonstrated superior efficacy in secondary processing of poultry and can inhibit pathogenic and aerobic bacteria sufficiently to add several days to the average shelf-life of refrigerated consumer poultry products.