Comparative Efficacy of Perasan MP-2 to Perasan MP-2C against *Campylobacter jejuni*

February 4, 2015
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Purpose

Perasan MP-2 is an FDA approved antimicrobial for use in the meat and poultry industry. The average concentration of hydrogen peroxide and peracetic acid is 5.5% and 15.2% respectively. Perasan MP-2C is a more concentrated form of the Perasan MP-2 with hydrogen peroxide and peracetic acid concentration of 4.7% and 22.8% respectively. The ratio of hydrogen peroxide to peracetic acid in the Perasan MP-2 is 0.36, while the ratio in Perasan MP-2C of hydrogen peroxide to peracetic acid is 0.21. While the ratio of peroxygens differs between the products, the efficacy of the product is due solely to the concentration of the peracetic acid – prepared solutions of similar peracetic acid concentrations will exhibit similar efficacies. The purpose of this study is to show that Perasan MP-2 and Perasan MP-2C exhibit similar efficacy against *Campylobacter jejuni* regardless of the ratio of hydrogen peroxide to peracetic acid in the two formulas.

Materials and Methods

Campylobacter jejuni bacteria (ATCC 33291) were cultured in Bolton Broth (Sigma Aldrich, lot number BCBB7257) containing 5% defibrinated sheep blood (Hardy Diagnostics) by anaerobic incubation for two days at 40° C. 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 four times to achieve a total of four *Campylobacter* inoculated Campy Cefex Agar plates. The plates were kept under anaerobic atmosphere and incubated for 48 hours at 40°C. After two days, the surfaces of the four *Campylobacter* inoculated Campy Cefex Agar plates were aseptically scraped using a sterile L-shaped and transferred to 650 mL of sterile water containing 2% chicken serum.

Preparation of Perasan MP-2 Stock Solution

Perasan MP-2 lot 825-012015-1 was analyzed at 15.50 % peracetic acid (PAA) and 5.87% hydrogen peroxide (H_2O_2) via iodometric titration. A 10,000 ppm PAA stock solution was prepared by diluting 5.83 mL of the Perasan MP-2 up to 100 mL in a Class A volumetric flask.

The stock solution was analyzed for PAA using the Palin DPD methodology and found to be 10,272 ppm PAA.

Preparation of Perasan MP-2C Stock Solution

Perasan MP-2C lot 844-011915-1 was analyzed at 22.47 % peracetic acid (PAA) and 5.11% hydrogen peroxide (H_2O_2) via iodometric titration. A 10,000 ppm PAA stock solution was prepared by diluting 4.00 mL of the Perasan MP-2C up to 100 mL in a Class A volumetric flask. The stock solution was analyzed for PAA using the Palin DPD methodology and found to be 10,379 ppm PAA.

Test Procedure

A total of three aliquots were aseptically removed from the 650 mL *C. jejuni* stock solution, serially diluted, and plated. These plates will serve as the control.

The remaining *C. jejuni* stock solution was divided into six 100mL samples. Three of the six bacterial samples were dosed with a nominal 80 ppm PAA by adding 800 µL of the Perasan MP-2 stock solution to each 100 mL sample. The concentration of PAA was check using the Palin DPD methodology immediately after dosing, then at 1 and 2 minutes after dosing. After 2 minutes 340 µL of a 10% erythorbic acid solution was used to neutralize any residual PAA and H_2O_2. Aliquots were taken from each sample, serially diluted, and plated on Campy Cefex Agar.

The remaining three *C. jejuni* bacterial solutions were dosed with a nominal 80 ppm PAA from Perasan MP-2C by dosing 700 µL of the Perasan MP-2C stock solution into each 100 mL sample. The concentration of PAA was checked using the Palin DPD methodology immediately after dosing, after 1 minute, and 2 minutes. After 2 minutes 340 µL of a 10% erythorbic acid solution was used to neutralize any residual PAA and H_2O_2. Aliquots were taken from each sample, serially diluted, and plated on Campy Cefex Agar.

All Campy Cefex Agar plates were incubated under anaerobic conditions for 48 hours at 40°C then enumerated.

Results and Discussion

Table 1 shows the average concentration of PAA in each test sample.

Description	Time (Minutes)	Average PAA Conc. (ppm)	Std. Dev. ppm
80 ppm Perasan MP-2	0	81.3	0.11
80 ppm Perasan MP-2	1	80.3	0.10
80 ppm Perasan MP-2	2	79.2	0.12
80 ppm Perasan MP-2C	0	79.6	0.13
80 ppm Perasan MP-2C	1	78.1	0.15
80 ppm Perasan MP-2C	2	79.2	0.11

Table 2 shows the average remaining *C. jejuni* remaining in the control samples and the test samples treated with 80 ppm PAA after 2 minutes.

Description	Avg. $\log_{10}$ Remaining (CFU/mL)	Std. Dev. (CFU/mL)	Avg. log10 Reduction (CFU/mL)	% Reduction
Control	5.49	0.33	NA	NA
80 ppm Perasan MP-2 (2 min)	0	0	>5.49	>99.999
80 ppm Perasan MP-2C (2 min)	0	0	>5.49	>99.999

The control samples had an average *Campylobacter jejuni* $\log_{10}$ of 5.49 ± 0.33 CFU/mL. After treatment with a nominal 80 ppm PAA from Perasan MP-2 or Perasan MP-2C in a 2% chicken serum solution there were zero remaining *Campylobacter jejuni* colonies remaining.

Conclusions

- Treatment with a nominal 80 ppm PAA from either Perasan MP-2 or Perasan MP-2C in a 2% chicken serum solution showed an average $\log_{10}$ of >5.49 CFU/mL in *Campylobacter jejuni* compared an untreated control sample.
- The results of this study show that the efficacy of Perasan MP-2 products is independent of the ratio of peroxygens present in the concentrate, as both products performed equally well against *Campylobacter jejuni* in a 2% chicken serum challenge solution.