The Efficacy of 300 ppm Peracetic Acid from Perasan MP-2 and MP-2C on *E. Coli* O157:H7 Inoculated Meat Surfaces

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Background

Enviro Tech's Food Contact Notification (FCN) 1132 allows the use of peracetic acid (PAA) up to 400 ppm to be applied to whole or cut meat including carcasses, parts, trim and organs. The purpose of this study is to determine the efficacy of 300 PAA from Perasan MP-2 (15% PAA) and Perasan MP-2C (22%) on *Escherichia coli* O157:H7 inoculated beef surfaces.

Materials and Methods

An Escherichia coli (ATCC® 35150) freeze-dried pellet was grown in Sigma Nutrient broth at 35°C for 48 hours. 1.0 mL aliquots were taken from the culture and plated on 15 x 100 mm Tryptic Soy Agar with 5% Sheep Blood (Hardy Diagnostics, Cat. No: A10). A total of 10 plates were cultured and incubated at 34.5°F for 36 hours. After, the bacteria were transferred to 5L of sterile Butterfield's Buffer via L-shaped plastic spreader. The inoculation solution was aseptically mixed to homogenize.

A total of 15 large beef round steaks were purchased from the local grocery store. The steaks were cut into equal pieces approximately 25 x 5 x 3 cm in length. A total of 20 strips were selected and the average mass was 263.3 ± 16.7 grams. The 20 meat samples were dipped in the prepared *E. coli* O157:H7 solution for 15 seconds. The strips of meat were then placed on a covered surface and allowed to sit undisturbed for 45 minutes to ensure bacterial attachment.

Perasan MP-2 (lot# 844-091516-1) was analyzed for peracetic acid and hydrogen peroxide via iodometric titration yielding concentrations of 15.21 and 5.83%, respectively. 115 L of a 300 ppm PAA solution was generated by diluting 226.97 grams of the Perasan MP-2 with 115 L of Modesto City water in a 55-gallon blue drum. The solution was analyzed for PAA using the Modified DPD methodology (Enviro Tech US Patent 7,651,860 B2) to yield concentrations of 299.6 ppm PAA.

Perasan MP-2C (lot# 844-092016-1) was analyzed for peracetic acid and hydrogen peroxide via iodometric titration yielding concentrations of 22.68 and 5.13%, respectively. 115 L of a 300 ppm PAA solution was generated by diluting 152.22 grams of the Perasan MP-2C with 115 L of Modesto City water in a 55-gallon blue drum. The solution was analyzed for PAA and hydrogen peroxide using the Modified DPD methodology (Enviro Tech US Patent 7,651,860 B2) to yield concentrations of 295.32 ppm PAA.

Typical meat processing facilities commonly treat beef and pork with antimicrobial solutions for approximately 60-120 seconds by spraying the beef and pork carcasses and trim with the solution in a spray cabinet. To simulate this process, a small spray cabinet was created for this study. A one inch air pump was used to deliver the test solution into half inch PVC tubing, which in turn allowed the test solutions to be dispensed out of six nozzles placed four and a half inches apart in a 30 gallon drum. Each

nozzle delivered 0.5 gpm of test solution. A regulator on the air pump was used to adjust the pressure of the spray as needed. The spray pressure can vary depending on the meat processing facility therefore, a low pressure of 20 psi was used in this experiment to simulate the lowest spray pressure that may be employed at a meat processing facility.

Five of the 20 strips of meat were placed in individual sterile stomacher bags along with 100 mL of sterile D/E Neutralizing Broth. The bags were agitated for 60 seconds to remove the surface bound *E. coli*. Next, aliquots were taken from each bag, serially diluted, and plated on 3M E. coli Petrifilms™. These samples would serve as the untreated control for the study.

Next, a 55-gallon drum containing only reverse osmosis water was connected to the spray cabinet. The air pump was turned on and the spray pressure was adjusted to 20 psi. Five strips of meat were chosen at random and were individually suspended using an 18-gauge stainless steel hook in the middle of the six spray nozzles for 60 seconds. After seconds, the meat strips were lifted from the spray cabinet and allowed to drip for an additional 10 seconds to allow excess liquid to drain. The strips were then placed into individual sterile stomacher bags along with 100 mL of sterile D/E Neutralizing Broth. The bag and contents were agitated for 60 seconds to remove surface bound E. coli. Aliquots were taken from each bag, serially diluted, and plated on 3M E. coli Petrifilms™. These samples would serve as the water treated control for the study

The 55-gallon drum containing 115 L of a nominal 300 ppm PAA solution from Perasan MP-2 was then connected to the spray cabinet. The air pump was turned on and the spray pressure was adjusted to 20 psi. Five remaining strips of meat were individually suspended using a 18 gauge stainless steel hook in the middle of the six spray nozzles for 60 seconds. After seconds, the meat strips were lifted from the spray cabinet and allowed to drip for an additional 10 seconds to allow excess liquid to drain. The strips were then placed into individual sterile stomacher bags along with 100 mL of sterile D/E Neutralizing Broth to neutralize any remaining peroxygen species. The bag and contents were agitated for 60 seconds to remove surface bound E. coli. Aliquots were taken from each bag, serially diluted, and plated on 3M E. coli Petrifilms™. These samples would serve as the PAA treated control for the study

The 55-gallon drum containing 115 L of a nominal 300 ppm PAA solution from Perasan MP-2C was then connected to the spray cabinet. The air pump was turned on and the spray pressure was adjusted to 20 psi. Five remaining strips of meat were individually suspended using a 18 gauge stainless steel hook in the middle of the six spray nozzles for 60 seconds. After seconds, the meat strips were lifted from the spray cabinet and allowed to drip for an additional 10 seconds to allow excess liquid to drain. The strips were then placed into individual sterile stomacher bags along with 100 mL of sterile D/E Neutralizing Broth to neutralize any remaining peroxygen species. The bag and contents were agitated for 60 seconds to remove surface bound E. coli. Aliquots were taken from each bag, serially diluted, and plated on 3M E. coli Petrifilms™. These samples would serve as the PAA treated control for the study

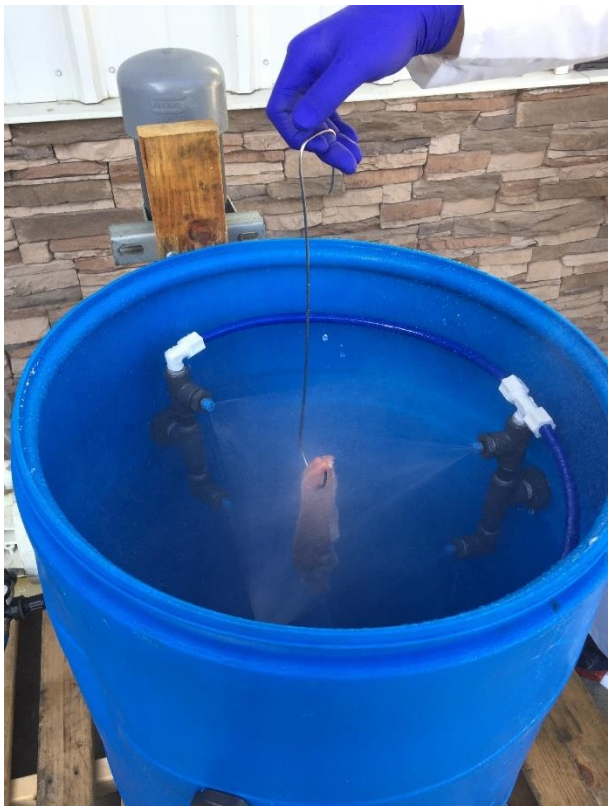
All Petrifilms™ were incubated at 35.0°F for 36 hours and enumerated.

Results

Table 1 shows the average remaining E. coli on the meat surfaces for the untreated, water treated, and 1200 ppm PAA treated samples.

Description	Avg. Remaining log_{10} (CFU/mL)	Avg. log_{10} Reduction	% Reduction	n
Control (Untreated)	6.60	NA	NA	5
Water Treated Only (60 sec.)	6.15	0.45	64.45	5
300 ppm PAA MP-2 Treated (60 sec.)	1.66	4.94	99.9989	5
300 ppm PAA MP-2C Treated (60 sec.)	1.64	4.96	99.9989	5

Imagine 1 shows the simulated spray cabinet and meat treating process.



After the meat strips were rinsed with the rinsed with the 100 mL of D/E Neutralizing Broth, the strips were rinse with tap water for 20 seconds and allowed to sit undisturbed for 20 minutes. The color and texture of the untreated, water treated, and 300 ppm PAA treated samples were compared. There appeared to be no difference in the different meat samples.

Image 2 shows the color and texture of an untreated meat strip

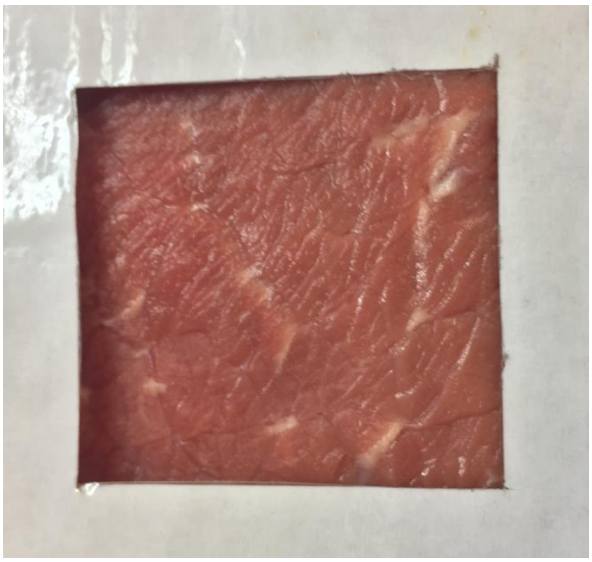


Image 3 shows the color and texture of a water treated meat strip

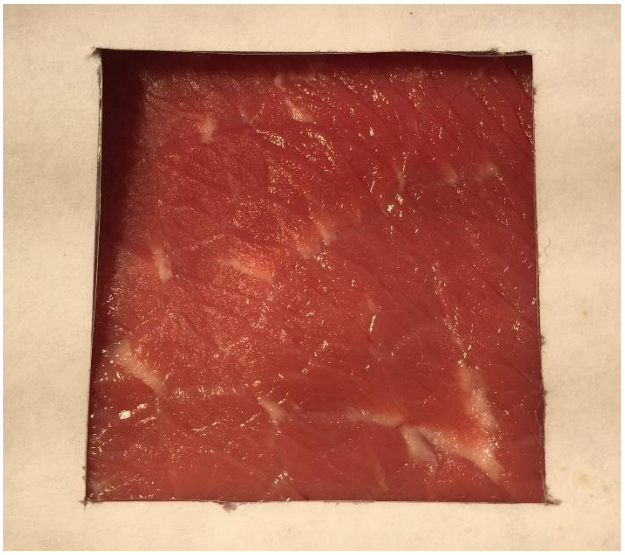
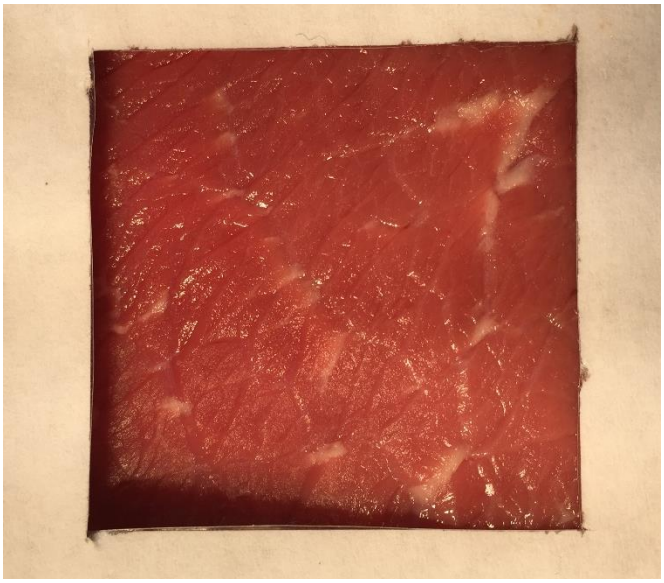


Image 4 shows the color and texture of a 1200 ppm treated meat strip



Conclusions

The untreated control meat samples contained an average E. coli log_{10} of 6.60 CFU/mL. After treatment with water only at 20 psi with a 60 second contact time, the remaining log_{10} was 6.15 CFU/mL (0.45-log reduction). Treatment with a nominal 300 ppm PAA from Perasan MP-2 and MP-2C for 60 seconds yielded very similar results with an average log_{10} reduction of 4.95 CFU/mL *E. coli* O157:H7 (>99.998% reduction). The appearance, color and texture, of the meat samples were essentially identical in the untreated, water treated, and 300 ppm PAA treated samples.