Efficacy of Peracetic Acid against Bacteria, Yeast, and Mold on Various Fruits and Vegetables

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Joseph Donabed, B.S.

Background

Currently the use of Enviro Tech Chemical Services' peroxyacetic acid (peracetic acid, PAA) on post-harvest fruits and vegetables is limited to 80 mg/L (ppm) as PAA per Food Contact Notification (FCN) 447 and 21 CFR 173.315(a)(5). Due to the ever-growing concern of food safety, more produce processing facilities are implementing antimicrobial intervention steps as well as conducting microbiological testing on their finished products. In the past, bacterial pathogens such as *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella spp*. were the main concern. However, produce processors are testing for yeast and mold species and finding that even at the maximum concentration with increased contact times; peracetic acid is failing to yield adequate reductions in higher lifeforms. The purpose of this study is to compare the efficacy of 80 ppm and 500 ppm PAA against various bacterial and yeast organisms.

Bacterial Organisms

Listeria monocytogenes

Listeria species are gram-positive, bacillus-shaped, soil dwelling bacteria. The major species that pertains to humans is *Listeria monocytogenes* which is a very adaptable and rugged bacterium and is the pathogen that is the causative agent for the disease listeriosis. Listeriosis is usually contracted by ingesting contaminated foods such as meat or dairy products. Prevention of listeriosis as a food illness is important and requires reliable and effective sanitation practices.

Salmonella typhimurium

Salmonella typhimurium is a pathogenic, gram-negative bacteria predominately found in the intestinal lumen of mammals. *Salmonella* typhimurium is a serovar of *Salmonella enterica* which has over 2500 different serovars. Six of the serovars are the most common human pathogens with *Salmonella* typhimurium being one of the six. The most common foods that can harbor *S.* typhimurium are; meat, poultry, produce, and raw eggs. Refrigerating or freezing does not destroy the bacteria but simply arrests growth. The hazardous nature of *S.* typhimurium is the presence of an outer membrane consisting largely of lipopolysaccharides (LPS) which protects the bacteria from the environment. Strong oxidizers such as PAA are able to penetrate the membrane and destroy *S.* typhimurium with great efficiency.

Escherichia coli O157:H7

Escherichia coli O157:H7 is a pathogenic serotype of *Escherichia coli* and is of the Shiga toxin generating *E. coli* serotypes. *Escherichia coli* O157:H7 is one of the most common pathogens associated with foodborne illness with transmission typically caused by fecal-oral route. Common contaminated foods are raw vegetables, meat, and dairy products.

Yeast and Mold Organisms

Candida albicans

Candida albicans is present in 80% of the population's normal flora without causing sickness but an overgrowth of *C. albicans* can lead to oral infection or even systemic infections. *C. albicans* is an opportunistic pathogen that usually affects immunocompromised individuals. *C. albicans* is very unique in that it is classified as a dimorphic organism meaning that it can grow either as yeast or mold. Since *C. albicans* can grow as yeast or mold, it has the ability to grow on many different substrates.

Saccharomyces cerevisiae

Saccharomyces cerevisiae is one the most common yeast species. *S. cerevisiae* is commonly used in wine and beer production, baking, and as a model organism in science. *S. cerevisiae* can be isolated from the skin of grapes as well as other dark skinned fruits such as plums, and thus fruit juices are more susceptible to *S. cerevisiae* contamination. Overgrowth of *S. cerevisiae* can lead to a severe fungal infection. Prevention of *S. cerevisiae* is important to in the preservation of food from spoilage as well as protecting consumers from foodborne illness. *S. cerevisiae* can be resistant to heat processing.

Aspergillus niger

Aspergillus niger is a very common fungus that can be found virtually anywhere. *A. niger* is the causative agent in a fruit and vegetable disease called "black mold". The most common fruits and vegetables affected are grapes, onions, and peanuts. *A. niger* is ubiquitous to the soil but can also be found in the environment. Fungal infections caused by *A. niger* in humans are not common but immune compromised individuals are susceptible to *A. niger* fungal infections. *A. niger* is more heat resistant to high temperatures and low water activity compared to other fungi which makes it dominate in warmer climates. Reduction or elimination of *Aspergillus niger* is important in preventing the spoilage of food.

Materials and Methods

Bacterial Organisms

Listeria monocytogenes (Hardy Diagnostic Cat# 0254FPC) was grown in Listeria Enrichment Broth (Criterion Cat. No.:C6030) and incubated at 35°C for 36 hours. The bacteria were separated from the broth by centrifugation. The liquid was decanted and the bacterial pellet was reconstituted in 2 L sterile phosphate buffer.

Salmonella typhimurium (ATCC® 14028) was grown in Sigma Nutrient broth at 35°C for 48 hours. The bacteria were separated from broth by centrifugation. The liquid was decanted and the bacterial pellet was reconstituted in 2 L sterile phosphate buffer.

Escherichia coli (ATCC® 35150) was grown in Sigma Nutrient broth at 35°C for 48 hours. The bacteria were separated from broth by centrifugation. The liquid was decanted and the bacterial pellet was reconstituted in 2 L sterile phosphate buffer.

Yeast and Mold Organisms

Candida albicans (ATCC® 10231) pellet was reconstituted in 10 mL of Brain Heart Infusion Broth (Criterion Cat. No.: C5140). After the pellet was completely dissolved, 1.0 mL aliquots of the solution was plated on 10 Hardy Diagnostic Sabdex Agar (Cat no.: W70) and the plates were incubated at 25°C for 72 hours. After 72 hours of incubation the Sabdex Agar plates had several 3-4 mm round, off-white colored colonies, which is indicative for *Candida albicans* growth. The yeast was aseptically transferred to 2 L of sterile Butterfield's Buffer which was then thoroughly mixed to ensure homogeneity.

Saccharomyces cerevisiae (ATCC® 18824) pellet was reconstituted in 10 mL of Criterion WL Nutrient Medium (Cat. No.: C7301). After the pellet was completely dissolved, 1.0 mL of the solution was plated on 10 Hardy Diagnostic Sabdex Agar (Cat no.: W70) after which the plates were incubated at 25 °C for 72 hours. After 72 hours of incubation, the Sabdex Agar plates had many 2-3 mm round, off-white colored colonies, which is indicative of *Saccharomyces cerevisiae* growth. The yeast was aseptically transferred to 2 L of sterile Butterfield's Buffer which was then thoroughly mixed to ensure homogeneity.

Aspergillus niger (ATCC® 16888) pellet was reconstituted in 10 mL of Brain Heart Infusion Broth (Criterion Cat. No.: C5140). After the pellet was completely dissolved, 1.0 mL of the solution was plated on five Hardy Diagnostic Sabdex Agar (Cat no.: W70) and the plates were incubated at 25 °C for 72 hours. After 72 hours of incubation the Sabdex Agar plates had several 4-5 mm filamentous white and black color colonies which are indicative of *Aspergillus niger* growth. The mold was aseptically transferred to 2 L of sterile Butterfield's Buffer which was then thoroughly mixed to ensure homogeneity.

A total of four different produce products were selected; whole apples (Red Delicious), whole potatoes (Russet), sliced tomatoes (Roma), and peeled carrots. The whole apples and potatoes would serve as the post-harvest, raw agricultural commodities (RACs) and the sliced tomatoes and peeled carrots would serve as the post-harvest non-RACs (further processed).

Preparation of Peracetic Acid Solutions

Two PAA solution concentrations were selected for this study: 80 ppm and 500 ppm in order to compare the current maximum allowed concentration, 80 ppm to the proposed concentration of 500 ppm. Perasan MP-2C (lot# 844-101016-1) was analyzed for peracetic acid and hydrogen peroxide (HP) via iodometric titration to yield concentrations of 22.08 and 5.14%, respectively. A fresh solution of 80 ppm and 500 ppm PAA was prepared fresh for each produce product tested by diluting 6.52 mL and 40.75 mL of the Perasan MP-2C to 20 mL Modesto city water, respectively. The nominal 80 ppm PAA and 18.6 ppm HP as well as the nominal 500 ppm PAA and 116.4 ppm HP solution was analyzed via Palin Modified DPD methodology and verified to be within 10% of the target concentrations.

Growth Media

Different growth mediums were used from the three different bacterial organism; 3M *E. coli* Petrifilms™, 3M *Enterobacteriaceae* Petrifilms™, and 3M *Listeria* Petrifilms™. *C. albicans*, *A. niger*, and *S. cerevisiae* were plated on Hardy Diagnostic's Sabdex Agar. All Petrifilms™ were incubated at 34.2°C for 28 hours and all Sabdex agar plates were incubated at 25.1°C for 96 hours.

Whole Apple Treatment

Approximately 65 pounds of Red Delicious apples were purchased from the local grocery. A total of 18 apples with each apple yielding an average mass of 250 g ± 15 grams were dipped in each of the six organism solution for 10 seconds. The apples were then placed on a covered surface and allowed to sit undisturbed for 45 minutes to ensure organism attachment. Three inoculated apples were selected from the six different inoculated sets and placed into individual sterile stomacher bags along with 50 mL of Dey-Engley (D/E) neutralizing broth. The bags were individually agitated for 30 seconds to ensure adequate removal of surface bound organisms. Aliquots were taken from each bag and plated on the appropriate growth media. These samples would serve as the untreated control samples.

Next, three inoculated apples from the six different organism group were submerged in 5 L of sterile reverse osmosis water for 60 seconds. The water solution was moderately stirred using a sterile stainless steel laboratory spatula. The apples were then transferred to individual sterile stomacher bags along with 50 mL of D/E neutralizing broth. The apples were agitated for 30 seconds to ensure adequate removal of surface bound organisms. Aliquots were taken from

each bag and plated on the appropriate growth media. These samples would serve as the water treated control samples.

Next, six apples from each organism group were submerged into separate 80 ppm and 500 ppm PAA solution and gently agitated using a sterile stainless steel laboratory spatula. After 30 seconds of contact, three apples from each solution were removed and aseptically transferred to individual sterile stomacher bags along with 50 mL of D/E neutralizing broth. After a total of 60 seconds, the remaining apples were transferred to individual sterile stomacher bags along with 50 mL of D/E neutralizing broth. The apples were agitated for 30 seconds to ensure adequate removal of surface bound organisms. Aliquots were taken from each bag and plated on the appropriate growth media. These samples would serve as the PAA treated samples.

Whole Potato Treatment

Approximately 100 pounds of Russet potatoes were purchased from the local grocery. A total of 36 potatoes were dipped in each of the six organism solution for 10 seconds. The average mass of two potatoes was 350 grams ± 15 grams. The potatoes were then placed on a covered surface and allowed to sit undisturbed for 45 minutes to ensure organism attachment. Six inoculated potatoes were selected from the six different inoculated sets and placed into individual sterile stomach bags (two potatoes per bag) along with 50 mL of Dey-Engley (D/E) neutralizing broth. The bags were individually agitated for 30 seconds to ensure adequate removal of surface bound organisms. Aliquots were taken from each bag and plated on the appropriate growth media. These samples would serve as the untreated control samples.

Next, six inoculated potatoes from each organism group were submerged in 5 L of sterile reverse osmosis water for 60 seconds. The water solution was moderately stirred using a sterile stainless steel laboratory spatula. The potatoes were then transferred to individual sterile stomacher bags (two potatoes per bag) along with 50 mL of D/E neutralizing broth. The potatoes were agitated for 30 seconds to ensure adequate removal of surface bound organisms. Aliquots were taken from each bag and plated on the appropriate growth media. These samples would serve as the water treated control samples.

Next, 12 potatoes from each organism group were submerged into separate 80 ppm and 500 ppm PAA solution and gently agitated using a sterile stainless steel laboratory spatula. After 30 seconds of contact, six potatoes from each solution were removed and aseptically transferred to individual sterile stomacher bags (two potatoes per bag) along with 50 mL of D/E neutralizing broth. After a total of 60 seconds, the remaining potatoes were transferred to individual sterile stomacher bags along with 50 mL of D/E neutralizing broth. The potatoes were agitated for 30 seconds to ensure adequate removal of surface bound organisms. Aliquots were taken from each bag, serially diluted, and plated on the appropriate growth media. These samples would serve as the PAA treated samples.

Sliced Tomatoes Treatment

Approximately 95 pounds of Roma tomatoes were purchased from three different local grocery stores. The tomatoes were sliced into 1/8 pieces. A total of 6400 grams of the sliced tomatoes were dipped in each of the six organism solutions for 10 seconds. The tomatoes were then placed on a covered surface and allowed to sit undisturbed for 45 minutes to ensure organism attachment. A total of 350 grams ± 13 grams of inoculated sliced tomatoes were selected from the six different inoculated sets and placed into individual sterile stomach bags along with 50 mL of Dey-Engley (D/E) neutralizing broth. This was repeated a total of three times per organism group. The bags were individually agitated for 30 seconds to ensure adequate removal of surface bound organisms. Aliquots were taken from each bag, serially diluted, and plated on the appropriate growth media. These samples would serve as the untreated control samples.

Next, 1100 grams of inoculated tomatoes from each organism group were submerged in 5 L of sterile reverse osmosis water for 60 seconds. The water solution containing the sliced tomatoes was moderately stirred using a sterile stainless steel laboratory spatula. A total of 350 grams of sliced tomatoes were then transferred to individual sterile stomacher bags along with 50 mL of D/E neutralizing broth. This was repeated in triplicate per organism group. The tomatoes were agitated for 30 seconds to ensure adequate removal of surface bound organisms. Aliquots were taken from each bag, serially diluted, and plated on the appropriate growth media. These samples would serve as the water treated samples.

Next, 2200 grams of inoculated tomatoes from each organism group were submerged into separate 80 ppm and 500 ppm PAA solution and gently agitated using a sterile stainless steel laboratory spatula. After 30 seconds of contact, 350 grams of sliced tomatoes from each solution were removed and aseptically transferred to individual sterile stomacher bags along with 50 mL of D/E neutralizing broth. This was repeated a total of three times per organism group. After a total of 60 seconds, the remaining tomatoes were transferred to individual sterile stomacher bags (350 grams sliced tomatoes per bag) along with 50 mL of D/E neutralizing broth. The tomatoes were agitated for 30 seconds to ensure adequate removal of surface bound organisms. Aliquots were taken from each bag, serially diluted, and plated on the appropriate growth media. These samples would serve as the PAA treated samples.

Peeled and Cut Carrot Treatment

Approximately 95 pounds of whole carrots were purchased from the local grocery store. The carrots were peeled using a standard kitchen peeler and were cut into 2 cm pieces. A total of 6400 grams of the peeled and cut carrots were dipped in each of the six organism solutions for 10 seconds. The carrots were then placed on a covered surface and allowed to sit undisturbed for 45 minutes to ensure organism attachment. A total of 350 grams ± 16 grams of inoculated peeled and cut carrots were selected from the six different inoculated sets and placed into individual sterile stomach bags along with 50 mL of Dey-Engley (D/E) neutralizing broth. This was repeated in triplicate per organism group. The bags were individually agitated for 30 seconds to

ensure adequate removal of surface bound organisms. Aliquots were taken from each bag, serially diluted, and plated on the appropriate growth media. These samples would serve as the untreated control samples.

Next, 1100 grams of inoculated carrots from each organism group were submerged in 5 L of sterile reverse osmosis water for 60 seconds. The water solution containing the peeled carrots was moderately stirred using a sterile stainless steel laboratory spatula. A total of 350 grams of carrot were then transferred to individual sterile stomacher bags along with 50 mL of D/E neutralizing broth. This was repeated in triplicate per organism group. The contents of the bag were agitated for 30 seconds to ensure adequate removal of surface bound microbes. Aliquots were taken from each bag, serially diluted, and plated on the appropriate growth media. These samples would serve as the water treated samples.

Next, 2200 grams of inoculated carrots from each organism group were submerged into separate 80 ppm and 500 ppm PAA solution and gently agitated using a sterile stainless steel laboratory spatula. After 30 seconds of contact, 350 grams of the peeled carrots from each solution were removed and aseptically transferred to individual sterile stomacher bags along with 50 mL of D/E neutralizing broth. This was repeated a total of three times per organism group. After a total of 60 seconds, the remaining carrots were transferred to individual sterile stomacher bags (350 grams carrots per bag) along with 50 mL of D/E neutralizing broth. The carrots were agitated for 30 seconds to ensure adequate removal of surface bound organisms. Aliquots were taken from each bag, serially diluted, and plated on the appropriate growth media. These samples would serve as the PAA treated samples.

Results

Figure 1 shows the comparative efficacy of a water wash (60 second contact time), 80 ppm PAA treatment (30 and 60 seconds), and 500 ppm PAA treatment (30 and 60 seconds) on untreated whole apples, whole potatoes, sliced tomatoes, and peeled carrots inoculated with *E. coli* O157:H7

Description	Avg. Remaining $\log_{10}$ (CFU/mL)	Avg. $\log_{10}$ Reduction (CFU/mL)	% Reduction	n
Whole Apples (Untreated Control)	6.53	NA	NA	3
Whole Apples (Water Control)	6.43	0.10	20.78	3
Whole Apples (80 ppm 30 sec.)	3.79	2.74	99.82	3
Whole Apples (80 ppm 60 sec.)	1.98	4.55	99.997	3
Whole Apples (500 ppm 30 sec.)	0.72	5.81	99.9998	3
Whole Apples (500 ppm 60 sec.)	<0.10	>6.43	>99.9999	3

Description	Avg. Remaining log_{10} (CFU/mL)	Avg. log_{10} Reduction (CFU/mL)	% Reduction	n
Whole Potatoes (Untreated Control)	6.68	NA	NA	3
Whole Potatoes (Water Control)	6.51	0.17	32.42	3
Whole Potatoes (80 ppm 30 sec.)	3.91	2.77	99.83	3
Whole Potatoes (80 ppm 60 sec.)	2.03	4.65	99.998	3
Whole Potatoes (500 ppm 30 sec.)	<0.10	>6.58	>99.9999	3
Whole Potatoes (500 ppm 60 sec.)	<0.10	>6.58	>99.9999	3

Description	Avg. Remaining log_{10} (CFU/mL)	Avg. log_{10} Reduction (CFU/mL)	% Reduction	n
Sliced Tomatoes (Untreated Control)	6.49	NA	NA	3
Sliced Tomatoes (Water Control)	6.38	0.11	22.29	3
Sliced Tomatoes (80 ppm 30 sec.)	3.93	2.56	99.72	3
Sliced Tomatoes (80 ppm 60 sec.)	1.82	4.67	99.998	3
Sliced Tomatoes (500 ppm 30 sec.)	<0.10	>6.39	>99.9999	3
Sliced Tomatoes (500 ppm 60 sec.)	<0.10	>6.39	>99.9999	3

Description	Avg. Remaining log_{10} (CFU/mL)	Avg. log_{10} Reduction (CFU/mL)	% Reduction	n
Peeled Carrots (Untreated Control)	6.63	NA	NA	3
Peeled Carrots (Water Control)	6.49	0.14	26.41	3
Peeled Carrots (80 ppm 30 sec.)	3.96	2.67	99.79	3
Peeled Carrots (80 ppm 60 sec.)	2.05	4.58	99.997	3
Peeled Carrots (500 ppm 30 sec.)	0.98	5.65	99.9998	3
Peeled Carrots (500 ppm 60 sec.)	<0.10	>6.53	>99.9999	3

Figure 2 shows the comparative efficacy of a water wash (60 second contact time), 80 ppm PAA treatment (30 and 60 seconds), and 500 ppm PAA treatment (30 and 60 seconds) on untreated whole apples, whole potatoes, sliced tomatoes, and peeled carrots inoculated with *S. typhimurium*.

Description	Avg. Remaining log_{10} (CFU/mL)	Avg. log_{10} Reduction (CFU/mL)	% Reduction	n
Whole Apples (Untreated Control)	6.47	NA	NA	3
Whole Apples (Water Control)	6.37	0.10	20.86	3
Whole Apples (80 ppm 30 sec.)	3.83	2.64	99.77	3
Whole Apples (80 ppm 60 sec.)	1.91	4.56	99.997	3
Whole Apples (500 ppm 30 sec.)	0.88	5.59	99.9997	3
Whole Apples (500 ppm 60 sec.)	<0.10	>6.37	>99.9999	3

Description	Avg. Remaining log_{10} (CFU/mL)	Avg. log_{10} Reduction (CFU/mL)	% Reduction	n
Whole Potatoes (Untreated Control)	6.61	NA	NA	3
Whole Potatoes (Water Control)	6.49	0.12	23.63	3
Whole Potatoes (80 ppm 30 sec.)	3.9	2.71	99.8	3
Whole Potatoes (80 ppm 60 sec.)	1.72	4.89	99.998	3
Whole Potatoes (500 ppm 30 sec.)	<0.10	>6.51	>99.9999	3
Whole Potatoes (500 ppm 60 sec.)	<0.10	>6.51	>99.9999	3

Description	Avg. Remaining log_{10} (CFU/mL)	Avg. log_{10} Reduction (CFU/mL)	% Reduction	n
Sliced Tomatoes (Untreated Control)	6.39	NA	NA	3
Sliced Tomatoes (Water Control)	6.35	0.04	8.39	3
Sliced Tomatoes (80 ppm 30 sec.)	3.94	2.45	99.65	3
Sliced Tomatoes (80 ppm 60 sec.)	1.84	4.55	99.997	3
Sliced Tomatoes (500 ppm 30 sec.)	<0.10	>6.29	>99.9999	3
Sliced Tomatoes (500 ppm 60 sec.)	<0.10	>6.29	>99.9999	3

Description	Avg. Remaining log_{10} (CFU/mL)	Avg. log_{10} Reduction (CFU/mL)	% Reduction	n
Peeled Carrots (Untreated Control)	6.71	NA	NA	3
Peeled Carrots (Water Control)	6.54	0.17	31.38	3
Peeled Carrots (80 ppm 30 sec.)	4.02	2.69	99.8	3
Peeled Carrots (80 ppm 60 sec.)	1.87	4.84	99.998	3
Peeled Carrots (500 ppm 30 sec.)	0.63	6.08	99.99992	3
Peeled Carrots (500 ppm 60 sec.)	<0.10	>6.61	>99.9999	3

Figure 3 shows the comparative efficacy of a water wash (60 second contact time), 80 ppm PAA treatment (30 and 60 seconds), and 500 ppm PAA treatment (30 and 60 seconds) on untreated whole apples, whole potatoes, sliced tomatoes, and peeled carrots inoculated with *Listeria monocytogenes*.

Description	Avg. Remaining log_{10} (CFU/mL)	Avg. log_{10} Reduction (CFU/mL)	% Reduction	n
Whole Apples (Untreated Control)	6.11	NA	NA	3
Whole Apples (Water Control)	5.93	0.18	33.78	3
Whole Apples (80 ppm 30 sec.)	3.50	2.61	99.75	3
Whole Apples (80 ppm 60 sec.)	<0.10	>6.01	>99.9999	3
Whole Apples (500 ppm 30 sec.)	<0.10	>6.01	>99.9999	3
Whole Apples (500 ppm 60 sec.)	<0.10	>6.01	>99.9999	3

Description	Avg. Remaining log_{10} (CFU/mL)	Avg. log_{10} Reduction (CFU/mL)	% Reduction	n
Whole Potatoes (Untreated Control)	6.78	NA	NA	3
Whole Potatoes (Water Control)	6.68	0.10	20.4	3
Whole Potatoes (80 ppm 30 sec.)	3.93	2.85	99.86	3
Whole Potatoes (80 ppm 60 sec.)	1.69	5.09	99.999	3
Whole Potatoes (500 ppm 30 sec.)	<0.10	>6.68	>99.9999	3
Whole Potatoes (500 ppm 60 sec.)	<0.10	>6.68	>99.9999	3

Description	Avg. Remaining log_{10} (CFU/mL)	Avg. log_{10} Reduction (CFU/mL)	% Reduction	n
Sliced Tomatoes (Untreated Control)	6.46	NA	NA	3
Sliced Tomatoes (Water Control)	6.39	0.07	15.27	3
Sliced Tomatoes (80 ppm 30 sec.)	3.85	2.61	99.75	3
Sliced Tomatoes (80 ppm 60 sec.)	1.59	4.87	99.998	3
Sliced Tomatoes (500 ppm 30 sec.)	<0.10	>6.36	>99.9999	3
Sliced Tomatoes (500 ppm 60 sec.)	<0.10	>6.36	>99.9999	3

Description	Avg. Remaining log_{10} (CFU/mL)	Avg. log_{10} Reduction (CFU/mL)	% Reduction	n
Peeled Carrots (Untreated Control)	6.59	NA	NA	3
Peeled Carrots (Water Control)	6.49	0.10	19.77	3
Peeled Carrots (80 ppm 30 sec.)	3.96	2.63	99.76	3
Peeled Carrots (80 ppm 60 sec.)	1.68	4.91	99.998	3
Peeled Carrots (500 ppm 30 sec.)	<0.10	>6.49	>99.9999	3
Peeled Carrots (500 ppm 60 sec.)	<0.10	>6.49	>99.9999	3

Figure 4 shows the comparative efficacy of a water wash (60 second contact time), 80 ppm PAA treatment (30 and 60 seconds), and 500 ppm PAA treatment (30 and 60 seconds) on untreated whole apples, whole potatoes, sliced tomatoes, and peeled carrots inoculated with *Aspergillus niger*.

Description	Avg. Remaining log_{10} (CFU/mL)	Avg. log_{10} Reduction (CFU/mL)	% Reduction	n
Whole Apples (Untreated Control)	5.89	NA	NA	3
Whole Apples (Water Control)	5.75	0.14	28.07	3
Whole Apples (80 ppm 30 sec.)	4.96	0.93	88.37	3
Whole Apples (80 ppm 60 sec.)	4.87	1.02	90.39	3
Whole Apples (500 ppm 30 sec.)	2.37	3.52	99.97	3
Whole Apples (500 ppm 60 sec.)	1.71	4.18	99.993	3

Description	Avg. Remaining log_{10} (CFU/mL)	Avg. log_{10} Reduction (CFU/mL)	% Reduction	n
Whole Potatoes (Untreated Control)	5.86	NA	NA	3
Whole Potatoes (Water Control)	5.73	0.13	26.69	3
Whole Potatoes (80 ppm 30 sec.)	5.07	0.79	83.74	3
Whole Potatoes (80 ppm 60 sec.)	2.88	2.98	99.896	3
Whole Potatoes (500 ppm 30 sec.)	2.04	3.82	99.985	3
Whole Potatoes (500 ppm 60 sec.)	1.53	4.33	99.9953	3

Description	Avg. Remaining log_{10} (CFU/mL)	Avg. log_{10} Reduction (CFU/mL)	% Reduction	n
Sliced Tomatoes (Untreated Control)	5.39	NA	NA	3
Sliced Tomatoes (Water Control)	5.30	0.09	15.27	3
Sliced Tomatoes (80 ppm 30 sec.)	3.79	1.60	99.75	3
Sliced Tomatoes (80 ppm 60 sec.)	3.67	1.72	99.998	3
Sliced Tomatoes (500 ppm 30 sec.)	1.66	3.73	99.981	3
Sliced Tomatoes (500 ppm 60 sec.)	1.32	4.07	99.991	3

Description	Avg. Remaining log_{10} (CFU/mL)	Avg. log_{10} Reduction (CFU/mL)	% Reduction	n
Peeled Carrots (Untreated Control)	5.32	NA	NA	3
Peeled Carrots (Water Control)	5.24	0.08	15.8	3
Peeled Carrots (80 ppm 30 sec.)	5.12	0.20	36.23	3
Peeled Carrots (80 ppm 60 sec.)	5.04	0.28	47.25	3
Peeled Carrots (500 ppm 30 sec.)	2.71	2.61	99.75	3
Peeled Carrots (500 ppm 60 sec.)	2.46	2.86	99.86	3

Figure 5 shows the comparative efficacy of a water wash (60 second contact time), 80 ppm PAA treatment (30 and 60 seconds), and 500 ppm PAA treatment (30 and 60 seconds) on untreated whole apples, whole potatoes, sliced tomatoes, and peeled carrots inoculated with *Candida albicans*.

Description	Avg. Remaining log_{10} (CFU/mL)	Avg. log_{10} Reduction (CFU/mL)	% Reduction	n
Whole Apples (Untreated Control)	5.54	NA	NA	3
Whole Apples (Water Control)	5.23	0.31	51.24	3
Whole Apples (80 ppm 30 sec.)	5.17	0.37	57.68	3
Whole Apples (80 ppm 60 sec.)	4.33	1.21	93.86	3
Whole Apples (500 ppm 30 sec.)	2.67	2.87	99.87	3
Whole Apples (500 ppm 60 sec.)	1.95	3.59	99.97	3

Description	Avg. Remaining log_{10} (CFU/mL)	Avg. log_{10} Reduction (CFU/mL)	% Reduction	n
Whole Potatoes (Untreated Control)	6.06	NA	NA	3
Whole Potatoes (Water Control)	5.85	0.21	37.5	3
Whole Potatoes (80 ppm 30 sec.)	5.55	0.51	68.79	3
Whole Potatoes (80 ppm 60 sec.)	5.02	1.04	90.79	3
Whole Potatoes (500 ppm 30 sec.)	2.49	3.57	99.97	3
Whole Potatoes (500 ppm 60 sec.)	1.72	4.34	99.995	3

Description	Avg. Remaining log_{10} (CFU/mL)	Avg. log_{10} Reduction (CFU/mL)	% Reduction	n
Sliced Tomatoes (Untreated Control)	5.28	NA	NA	3
Sliced Tomatoes (Water Control)	4.89	0.39	59.57	3
Sliced Tomatoes (80 ppm 30 sec.)	4.65	0.63	76.73	3
Sliced Tomatoes (80 ppm 60 sec.)	3.76	1.52	97.00	3
Sliced Tomatoes (500 ppm 30 sec.)	2.89	2.39	99.60	3
Sliced Tomatoes (500 ppm 60 sec.)	1.83	3.45	99.96	3

Description	Avg. Remaining log_{10} (CFU/mL)	Avg. log_{10} Reduction (CFU/mL)	% Reduction	n
Peeled Carrots (Untreated Control)	5.52	NA	NA	3
Peeled Carrots (Water Control)	4.93	0.59	74.3	3
Peeled Carrots (80 ppm 30 sec.)	4.73	0.79	83.78	3
Peeled Carrots (80 ppm 60 sec.)	4.21	1.31	95.10	3
Peeled Carrots (500 ppm 30 sec.)	3.45	2.07	99.15	3
Peeled Carrots (500 ppm 60 sec.)	2.13	3.39	99.96	3

Figure 6 shows the comparative efficacy of a water wash (60 second contact time), 80 ppm PAA treatment (30 and 60 seconds), and 500 ppm PAA treatment (30 and 60 seconds) on untreated whole apples, whole potatoes, sliced tomatoes, and peeled carrots inoculated with *Saccharomyces cerevisiae*.

Description	Avg. Remaining log10 (CFU/mL)	Avg. log10 Reduction (CFU/mL)	% Reduction	n
Whole Apples (Untreated Control)	5.57	NA	NA	3
Whole Apples (Water Control)	5.49	0.08	16.84	3
Whole Apples (80 ppm 30 sec.)	4.09	1.48	96.69	3
Whole Apples (80 ppm 60 sec.)	3.55	2.02	99.06	3
Whole Apples (500 ppm 30 sec.)	2.51	3.06	99.91	3
Whole Apples (500 ppm 60 sec.)	1.84	3.73	99.98	3

Description	Avg. Remaining log10 (CFU/mL)	Avg. log10 Reduction (CFU/mL)	% Reduction	n
Whole Potatoes (Untreated Control)	5.04	NA	NA	3
Whole Potatoes (Water Control)	4.91	0.13	24.69	3
Whole Potatoes (80 ppm 30 sec.)	4.7	0.34	53.75	3
Whole Potatoes (80 ppm 60 sec.)	4.37	0.67	78.40	3
Whole Potatoes (500 ppm 30 sec.)	2.74	2.30	99.50	3
Whole Potatoes (500 ppm 60 sec.)	1.08	3.96	99.98	3

Description	Avg. Remaining log10 (CFU/mL)	Avg. log10 Reduction (CFU/mL)	% Reduction	n
Sliced Tomatoes (Untreated Control)	5.68	NA	NA	3
Sliced Tomatoes (Water Control)	5.60	0.08	15.16	3
Sliced Tomatoes (80 ppm 30 sec.)	4.56	1.12	92.27	3
Sliced Tomatoes (80 ppm 60 sec.)	2.51	3.17	99.93	3
Sliced Tomatoes (500 ppm 30 sec.)	2.03	3.65	99.97	3
Sliced Tomatoes (500 ppm 60 sec.)	0.95	4.73	99.998	3

Description	Avg. Remaining log10 (CFU/mL)	Avg. log10 Reduction (CFU/mL)	% Reduction	n
Peeled Carrots (Untreated Control)	5.44	NA	NA	3
Peeled Carrots (Water Control)	5.39	0.05	12.39	3
Peeled Carrots (80 ppm 30 sec.)	4.97	0.47	66.73	3
Peeled Carrots (80 ppm 60 sec.)	4.75	0.69	70.78	3
Peeled Carrots (500 ppm 30 sec.)	2.01	3.43	99.96	3
Peeled Carrots (500 ppm 60 sec.)	1.01	4.43	99.996	3

Organoleptic Properties

In addition to efficacy, the effects of the treatment of a 500 ppm PAA solution for 60 seconds on the appearance of the RAC produce and non-RAC produce were compared to the untreated, water treated, and 80 ppm PAA treated samples. After the produce samples were rinsed with the D/E neutralizing broth and the solution plated for quantification of microbes, the produce samples were rinsed for approximately 30 seconds and air-dried for 60 minutes. A single sample was chosen at random from untreated, water treated, 80 ppm PAA treated, and 500 ppm PAA treated groups and the visual appearance was compared. There appeared to be no changes in

the appearance of the produce such as loss of color, whitening/darkening of the visible portion, nor any gross indicator that would differentiate the treated sample to the untreated sample.

Image 1 compares apples (left to right) from the untreated, water treated, 80 ppm PAA treated, and 500 ppm PAA treated samples

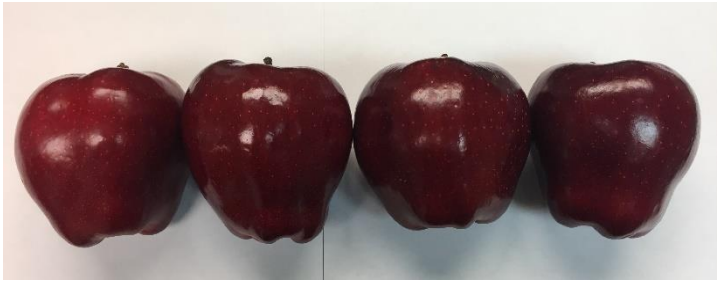


Image 2 compares whole potatoes (left to right) from the untreated, water treated, 80 ppm PAA treated, and 500 ppm PAA treated samples.

Image 3 compares sliced tomatoes (left to right) from the untreated, water treated, 80 ppm PAA treated, and 500 ppm PAA treated samples.

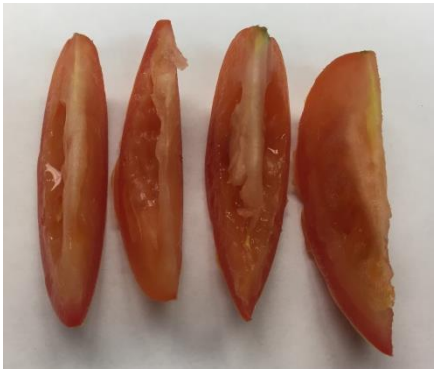
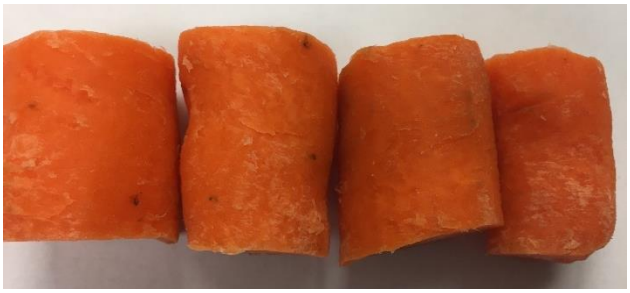


Image 4 compares peeled and cut carrots (left to right) from the untreated, water treated, 80 ppm PAA treated, and 500 ppm PAA treated samples.



Conclusion

The results of this study show that while treatment with 80 ppm peracetic acid from the Perasan MP-2C may achieve adequate bacterial reductions, it fails to achieve significant log_{10} reductions in yeast and mold organisms. Treatment with 500 ppm PAA from Perasan MP-2C with a 60 second contact time yielded significant reductions in yeast and mold organisms with the minimum reduction being against *Aspergillus niger* inoculated peeled carrots achieving a modest 2.86-log_{10} reduction. Treatment with 500 ppm PAA from Perasan MP-2C with a 60 second contact time completely eliminated *E.coli*, *Salmonella typhimurium*, and *Listeria monocytogenes* from the surfaces of the RAC and non-RAC produce. Lastly, treatment with 500 ppm PAA did not lead to any visual change in the appearance of the produce samples. Overall, the results of this study suggest that treatment with 500 ppm PAA on various produce products significantly increases overall efficacy against yeast and mold organisms compared to the current maximum of 80 ppm PAA. These findings are important for producers in the pursuit of increasing the safety of their products. Treatment up to 500 ppm PAA is allowed using the Perasan MP-2C under FCN 1738.