Antimicrobial Efficacy of a Peroxyacetic/Octanoic Acid Mixture in Fresh-Cut-Vegetable Process Waters

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ABSTRACT: The antimicrobial efficacy of a peroxyacetic/octanoic acid mixture for fruit and vegetable process water was compared to peroxyacetic acid alone. Rate-of-kill studies using yeast and molds indicated that the peroxyacetic/octanoic acid mixture had greater antifungal efficacy. In tests conducted during vegetable processing, use of the peroxyacetic/octanoic acid mixture was associated with lower numbers of yeast and molds in process water. In 1 example, lower numbers of yeast and molds in potato processing water containing the peroxyacetic acid/octanoic mixture were associated with lower yeast and mold counts on potatoes. Peroxyacetic/octanoic acid mixtures may offer processors using peroxyacetic acid a way to improve fungal reduction in recycled water processes.

Key Words: peracid, fresh-cut, vegetable, antimicrobial, antifungal

Introduction

WASHING FRESH PRODUCE WITH WATER can reduce potential contamination, however wash water may also serve as a source of contamination or cross-contamination (FDA 1998). If pathogenic microorganisms in water are not removed, inactivated, or otherwise controlled, they can spread to surrounding produce, potentially contaminating them (Tauxe and others 1997). Left untreated, recycled water tends to clean produce early in a shift but contaminates produce later in the shift (Brackett 1992). Flume water has been identified as a potential source of coliform, *E. coli*, and *Salmonell*a contamination or cross-contamination during cider production (Buchanan and others 1999).

The addition of antimicrobial agents to recycled process water can inactivate bacterial and fungal cells in water, helping avoid cross-contamination (Nguyen-the and Carlin 1994; Bartz 1999). The application of hypochlorites (Adams and others 1989; Zhuang and others 1995; Escudero and others 1999), chlorine dioxide (Zhang and Farber 1996), and peroxygen compounds (Nguyen-the and Carlin 1994; Sapers and Simmons 1998) helps reduce microbe populations on the surface of fresh produce.

An area of specific interest to fruit and vegetable processors is performance of process water antimicrobial agents against yeast and molds. Reduction of yeast and molds on processed vegetables, particularly those used as ingredients in ready-to-eat foods, is desired to improve finished-product quality and to prolong shelf life. A difficulty encountered in achieving this goal is that most fungi are intrinsically less susceptible to biocides than are bacteria. Increasing biocide concentration or exposure time to improve fungicidal activity may not always be possible due to destructive changes on the fruit or vegetable.

A composition containing octanoic acid and peroxyacetic acid was recently introduced to fruit and vegetable processors as a more potent fungicide than peroxyacetic acid. This study compared the efficacy of the octanoic- and peroxyacetic-acid mixture to peroxyacetic acid alone. Researchers first measured the antifungal potency of both chemistries in aqueous systems through laboratory rate-of-kill testing. The aforementioned testing was performed using fungi harvested from fresh produce. Later, examinations performed during fresh-cut vegetable processing served to evaluate microbial counts in wash water and on vegetable surfaces exposed to peracid-treated waters.

Materials and Methods

Preparation of fungi for rate-of-kill testing

Routine transfer to Sabouraud Dextrose agar slants maintained cultures of *Candida parapsilosis* (from blueberry processing water), *Rhodotorula* species (from celery processing water), *Cryptococcus* species (from potato processing water), and *Zygosaccharomyces bailii* (ATCC 60483). Growth was harvested by adding 5 mL phosphate-buffered water to the slant, mixing, and then transferring the suspension into 90 mL phosphate-buffered water. The resulting suspension was used for testing.

Routine transfer to Sabouraud Dextrose agar slants maintained cultures of *Aspergillus* species (from onion processing water), *Penicillium* species (from celery processing water), and *Cladosporium* species (from potato processing water). Conidia suspensions of each mold were prepared by subculturing each mold to Sabouraud Dextrose agar plates and incubating until thick aerial mycelia with conidia were evident. Conidia were harvested by adding approximately 10 mL phosphate-buffered water to the plate, scraping the mycelia, and collecting the suspension in a flask. The suspension was filtered through sterile gauze to remove large mycelia fragments from the conidia. The resulting suspension was used for testing.

The density of the fungal suspensions was determined by preparing serial 10-fold dilutions of the suspensions in phosphate-buffered water and pour-plating using Sabouraud Dextrose agar. Agar plates were incubated for 72 h at 26 °C before counting fungal colonies.

Rate of fungus kill

The rate at which peroxyacetic acid and the peroxyacetic/octanoic acid mixture killed fungi was measured by inoculating working solutions of each antimicrobial agent with fungi and then quantifying survivors after various exposure times.

Testing was performed in duplicate. Ninety-nine mL of each working solution or a phosphate-buffered water control was transferred to a 250-mL Erlenmeyer flask and allowed to equilibrate to 25 °C. The liquid in the flask was swirled vigorously in a rapid circular motion, and 1 mL of a fungus suspension was added. After 30 s, 2 min, 5 min, or 10 min, 1-mL quantities of the working solution or water control were transferred to 9 mL of an inactivating solution consisting of 0.1% sodium thiosulfate. One-mL quantities of the working solution or water control in the inactivating solution were pour-plated using Sabouraud Dextrose agar. Serial 100-fold dilutions of the working solution or water control in the inactivating solution were also prepared and plated. Agar plates were incubated for 72 h at 26 °C before counting survivors. The Log reduction of fungi due to the antimicrobial agent was determined by comparison to the water control.

Preparation of antimicrobial agents for rate-of-kill testing

Two antimicrobial agents were used for testing. The 1st agent contained peroxyacetic acid (Tsunami® 100, Ecolab Inc., St. Paul, Minn., U.S.A.). The 2nd contained a peroxyacetic/octanoic acid mixture (Tsunami® 200, Ecolab Inc.). The concentration of peroxyacetic, octanoic, and peroxyoctanoic acid in each agent was determined using high-pressure liquid chromatography (HPLC). Based on HPLC results, both agents were diluted in sterile deionized water to achieve working solutions with a total peracid concentration of 80 ppm. After preparing working solutions, the concentration of total peracid was verified by an iodine-sodium thiosulfate redox titration. The composition of the working solutions were as follows (see box below):

Chemical composition of working solutions used for rate-of-kill testing

Chemical	Peroxyacetic Acid Working Solution	Peroxyacetic/Octanoic Acid Working Solution
Peroxyacetic Acid	80 ppm	64 ppm
Octanoic Acid	None	53 ppm
Peroxyoctanoic Acid	None	16 ppm

Antimicrobial efficacy in vegetable-processing water

The reduction of microorganisms in vegetable-processing water treated with peroxyacetic acid or a peroxyacetic/octanoic acid mixture was determined. Testing was done in a commercial vegetable-processing facility using 2 separate recycled-water systems. In both systems, cut vegetables fell into a water stream that carried them to a de-watering area where they were collected for further processing. The water that the vegetables traveled in was routed to a balance tank that fed back into the original water stream, thus completing the process loop. One system was used to wash cut, raw celery or cabbage. The other system was used to transport cut, raw potatoes to a blancher.

During processing of each vegetable, 10 water samples were collected in sterile plastic bags containing an inactivating agent (0.01% sodium thiosulfate). One-mL quantities were plated on Petrifilm® (3M, Inc., St. Paul, Minn., U.S.A.) Aerobic Plate Count, *E. coli*/Coliform Count, and Yeast and Mold Count media. Validation of these media using this procedure was performed prior to initiating testing (data not presented). Serial 100-fold dilutions were also plated. Petrifilms were incubated at 35 °C for 24 h (Coliform Count), 35 °C for 48 h (Aerobic Plate Count), or at room temperature for 72 h (Yeast and Mold Count). The number of microorganisms was counted following incubation.

Concentration of antimicrobial agents in fresh-cut-vegetable processing water

Vegetable-processing water testing was done using the same antimicrobial agents as in laboratory rate-of-kill testing. The target concentration range of total peracid in the process water was 30 to 40 ppm. Concentrations of total peracid in process waters were determined by iodine-sodium thiosulfate redox titration. The goal was to test the effects of both the peroxyacetic acid and peroxyacetic/octanoic acid agents at approximately the same total peracid concentration. The concentrations encountered during testing are listed in the result section. Municipal tap water was the diluent.

Antimicrobial efficacy on fresh-cut vegetables

Microorganism reduction on vegetable surfaces in the process water was determined while water analyses were being conducted. Ten samples of each cut vegetable were collected just prior to falling into the process-water stream and just after de-watering.

Eleven grams of each sample were transferred to a bag containing 99 mL of sterile phosphate-buffered water. Contents of the bag were mixed in a Stomacher-brand mixer for approximately 1 min. Afterwards, 1 mL of the mixture was plated on Petrifilm brand Aerobic Plate Count, *E. coli*/Coliform Count, and Yeast and Mold Count media. One-mL quantities of 100-fold serial dilutions were also plated. Petrifilms were incubated and enumerated as described previously

Statistical analyses

A single-factor analysis of variance (ANOVA) using Microsoft Excel software was used to determine if microorganism numbers (Log_{10} transformed) in process water treated with the peroxyacetic/octanoic acid mixture were significantly lower than those with peroxyacetic acid. At an alpha level of 0.05, if results showed that $P \leq 0.05$, a significant lowering was concluded. The same analysis was used to evaluate the significance of reduction on vegetable surfaces.

Results and Discussion

Rate of fungus kill

The starting populations of *Candida parapsilosis, Rhodotorula* ssp., *Cryptococcus* ssp., and *Zygosaccharomyces bailii* in suspension were 7.30, 4.88, 8.08, and 5.98 Log_{10} CFU/mL, respectively. The peroxyacetic/octanoic acid mixture reduced the number of *Candida parapsilosis* and *Zygosaccharomyces bailii* cells in suspension faster than the peroxyacetic-acid agent (Table 1). No difference in reduction of *Rhodotorula* or *Cryptococcus* sp. was detected.

The starting populations of *Aspergillus* sp., *Penicillium* sp., and *Cladosporium* sp. were 6.28, 6.45, and 5.18 Log_{10} CFU/mL, respectively. The peroxyacetic/octanoic acid mixture reduced the number of mold conidia in suspension faster than the peroxyacetic-acid agent (Table 2).

Antimicrobial efficacy in fresh-cut-vegetable processing water

Microbial loads on fresh-cut vegetables entering the peroxyacetic acid or peroxyacetic/octanoic acid-containing water were not significantly different ($P \leq 0.05$) (Table 3). The concentrations of total peracid in the celery- and cabbage-processing waters were not significantly different ($P \leq 0.05$). The concentration of total peracid from the peroxyacetic-acid agent in the potato-processing water was approximately 20 ppm greater than the total peracid concentration from the peroxyacetic/octanoic acid mixture. When either antimicrobial agent was present in the processing water, coliform-bacteria levels were less than 1 Log CFU/mL (Table 4). The celery- and cabbage-process waters treated with the peroxyacetic/octanoic acid mixture showed significantly lower numbers of total aerobic bacteria than peroxyacetic-acid-treated water ($P \leq 0.05$). No significant difference in total bacteria numbers was observed in the potato-processing water. All process waters treated with the peroxyacetic/octanoic acid mixture showed significantly lower numbers of yeast and mold than peroxyacetic-acid-treated water ($P \leq 0.05$). The typical microorganism numbers that would have been encountered in the process waters had they been left untreated during processing were unknown. It is possible that some coliform bacteria, yeast, and molds may have been sublethally injured by the antimicrobial agents and thus not detected on the selective media used for enumeration.

Table 1—Average (n = 2) log reduction of yeast by peroxyacetic acid (POAA) and a peroxyacetic/octanoic acid (POAA/OA) mixture

	30 S		2 Min		5 Min	
	POAA[a]	POAA/OA[b]	POAA	POAA/OA	POAA	POAA/OA
Candida parapsilosis	0.34	3.49	1.49	3.46	3.42	4.12
Rhodotorula ssp.	> 3.88	> 3.88	> 3.88	> 3.88	> 3.88	> 3.88
Cryptococcus ssp.	> 7.08	> 7.08	> 7.08	> 7.08	>7.08	> 7.08
Zygosaccharomyces bailii	0.16	0.42	0.18	4.32	0.94	4.80

[a] POAA working solutions contained 80 ppm peroxyacetic acid.
[b] POAA/OA working solutions contained 64 ppm peroxyacetic acid, 16 ppm peroxyoctanoic acid, 53 ppm octanoic acid.

Table 2—Average (n = 2) log reduction of mold by peroxyacetic acid (POAA) and a peroxyacetic/octanoic acid (POAA/OA) mixture

	2 Min		5 Min		10 Min	
	POAA[a]	POAA/OA[b]	POAA	POAA/OA	POAA	POAA/OA
Aspergillus ssp.	0	0.13	0	1.60	0	2.13
Penicillium ssp.	0	4.19	0.45	4.79	1.52	5.45
Cladosporium ssp.	1.86	> 4.18	4.18	> 4.18	> 4.18	> 4.18

[a] POAA working solutions contained 80 ppm peroxyacetic acid.
[b] POAA/OA working solutions contained 64 ppm peroxyacetic acid, 16 ppm peroxyoctanoic acid, 53 ppm octanoic acid.

Table 3—Average number (Log CFU/g) of microorganisms on fresh-cut vegetables before entering process water containing peroxyacetic acid (POAA) or a peroxyacetic/octanoic acid (POAA/OA) mixture

	Aerobic Plate Count		Coliform Count		Yeast & Mold Count	
	Before Entering POAA Water	Before Entering POAA/OA Water	Before Entering POAA Water	Before Entering POAA/OA Water	Before Entering POAA Water	Before Entering POAA/OA Water
Celery	4.48 ± 0.57 A[a]	4.28 ± 0.60 A	3.83 ± 0.82 A	3.59 ± 0.31 A A	3.16 ±0.28 A	3.48 ± 0.20 A
Cabbage	3.31 ± 0.88 A	2.98 ± 0.76 A	2.23 ± 0.48 A	2.03 ± 0.52 A	2.86 ± 0.24 A	2.41 ± 0.64 A
Potatoes	4.36 ± 0.57 A	4.42 ± 0.45 A	3.68 ± 0.90 A	3.41 ± 0.82 A	1.63 ± 0.42 A	1.23 ± 0.21 A

[a] Averages (n = 10; ± SD) in the same row for the same microorganisms (Aerobic Plate Count, Coliform Count, Yeast and Mold Count) followed by the same letter (A or B) are not different (P > 0.05).

Table 4—Average number (Log CFU/mL) of microorganisms in fresh-cut-vegetable process water containing peroxyacetic acid (POAA) or a peroxyacetic/octanoic acid (POAA/OA) mixture

	Aerobic Plate Count		Coliform Count		Yeast & Mold Count	
	POAA	POAA/OA	POAA	POAA/OA	POAA	POAA/OA
Celery[a]	2.02 ± 0.05 A[d]	1.21 ± 0.29 B	< 1[e]	< 1	1.86 ± 0.14 A	0.32 ± 0.31 B
Cabbage[b]	1.92 ± 0.06 A	1.78 ± 0.08 B	< 1	< 1	2.02 ± 0.07 A	0.97 ± 0.33 B
Potatoes[c]	0.92 ± 0.32 A	0.95 ± 0.51 A	< 1	< 1	5.48 ± 0.11 A	2.67 ± 0.60 B

[a] Average concentration of peracid (n = 10; ± SD) was 38 ± 4 ppm (POAA agent), 39 ± 3 ppm (POAA/OA agent).
[b] Average concentration of peracid (n = 10; ± SD) was 26 ± 5 ppm (POAA agent), 29 ± 3 ppm (POAA/OA agent).
[c] Average concentration of peracid (n = 10; ± SD) was 54 ± 10 ppm (POAA agent), 32 ± 6 ppm (POAA/OA agent).
[d] Averages (n = 10; ± SD) in the same row for the same microorganisms (Aerobic Plate Count, Coliform Count, Yeast and Mold Count) followed by the same letter (A or B) are not different (P > 0.05).
[e] All samples had counts lower than the test detection limit of 1 Log CFU/mL.

Table 5—Average log reduction of microorganisms on fresh-cut vegetables after 30-s exposure to process water containing peroxyacetic acid (POAA) or a peroxyacetic/octanoic acid (POAA/OA) mixture

	Aerobic Bacteria Reduction		Coliform Bacteria Reduction		Yeast & Mold Reduction	
	POAA	POAA/OA	POAA	POAA/OA	POAA	POAA/OA
Celery[a]	1.07 ± 0.74 A[d]	1.10 ± 0.57 A	0.78 ± 0.84 A	1.41 ± 0.65 B	0.69 ± 0.44 A	0.96 ± 0.20 A
Cabbage[b]	0.84 ± 0.80 A	0.85 ± 0.52 A	0.84 ± 0.49 A	0.23 ± 0.63 A	0.77 ± 0.33 A	0.82 ± 0.40 A
Potatoes[c]	1.54 ± 0.63 A	1.69 ± 1.03 A	1.58 ±1.04 A	1.66 ± 0.91 A	No reduction, count increase of 2.88 ± 0.44 A	No reduction, count increase of 0.83 ± 0.43 B

[a] Average concentration of peracid (n = 10; ±SD) was 38 ±4 ppm (POAA agent), 39 ± 3 ppm (POAA/OA agent).
[b] Average concentration of peracid (n = 10; ±SD) was 26 ± 5 ppm (POAA agent), 29 ± 3 ppm (POAA/OA agent).
[c] Average concentration of peracid (n = 10; ±SD) was 54 ±10 ppm (POAA agent), 32 ± 6 ppm (POAA/OA agent).
[d] Averages (n = 10; ± SD) in the same row for the same microorganisms (Aerobic Plate Count, Coliform Count, Yeast and Mold Count) followed by the same letter (A or B) are not different (P > 0.05).

Antimicrobial efficacy on fresh-cut vegetables

The peroxyacetic/octanoic acid mixture showed significantly greater reductions in coliform bacteria on celery than peroxyacetic acid ($P \leq 0.05$) (Table 5). On potatoes, use of the peroxyacetic/octanoic acid mixture resulted significantly lower numbers of yeast and molds ($P \leq 0.05$). Some coliform bacteria, yeast, and molds may have been sublethally injured by the antimicrobial agents and thus not detected on the selective media used for enumeration. No other significant differences in log reduction were observed on processed vegetables.

Discussion

LABORATORY RATE-OF-KILL TESTING RESULTS corroborate existing research demonstrating that peroxyacetic/octanoic acid mixtures have greater antifungal potency than peroxyacetic acid (Cords 1994). Based on this knowledge, it was not surprising that yeast and mold counts in fresh-cut-vegetable process water containing the peroxyacetic/octanoic acid mixture were significantly lower than water containing peroxyacetic acid. Microorganisms transferred from vegetable surfaces into wash water have little physical protection and are susceptible to antimicrobial agents. Conversely, bacteria on vegetable tissues often have unique physical barriers preventing their direct contact with antimicrobial agents in wash water (Carmichael and others 1999; Seo and Frank 1999). Senter and others (1985) showed that chlorine treatment had little effect on surface microflora of tomatoes and oranges during packing operations. Brackett (1992) concluded that commonly used antimicrobial agents have only minor effects and should not be relied upon to eliminate microorganisms from produce. This problem of microorganism accessibility is probably why significant differences in microorganism counts were more prevalent in process-water tests than vegetable-surface tests.

Use of the peroxyacetic/octanoic acid

mixture in cut-potato flume water, instead of peroxyacetic acid, was indicated. High numbers of yeast and mold in process water containing peroxyacetic acid (5.48 Log CFU/mL) were associated with high numbers of yeast and molds on cut potatoes after exposure to the water. The significant lowering of yeast and mold counts on cut potatoes that occurred when peroxyacetic acid was replaced by the peroxyacetic/octanoic acid mixture was likely due to the corresponding reduction of yeast and molds in flume water. In this scenario, the yeast and mold numbers on the cut potatoes immersed in the peroxyacetic-acid-containing water were being increased as a result of cross-contamination. The improved count reductions observed with potatoes occurred despite testing the peroxyacetic/octanoic acid mixture at approximately 60% the total peracid concentration used in peroxyacetic-acid tests.

The use of peroxyacetic acid is sometimes preferred over other commonly used antimicrobial agents such as chlorine and hypochlorite because this agent has environment-friendly decomposition by-products (oxygen, acetic acid, water) and exhibits greater stability in the presence of organic soils. Peroxyacetic/octanoic acid mixtures also possess these beneficial attributes, octanoic acid being an additional decomposition by-product. Because of the relative instability of chlorine, it is common for processors to chlorinate water at 100 to 300 ppm available chlorine. Such concentrations of available chlorine are well above the minimum lethal dose for most bacteria (Bartz 1999). Excessive chlorination of food-processing water with hypochlorite has prompted concern over production of harmful organochlorine compounds. Chloroform, N-chloro compounds, chlorinated purine and pyrimidines, chlorophenols, and chlorobenzenes are some of the carcinogenic and toxic compounds resulting from chlorination of food products and water (Cheng-I and others 1985; Fukayama and others 1986).

Conclusions

COMBINATIONS OF OCTANOIC AND peroxyacetic acid may provide vegetable processors currently using peroxyacetic acid with reduced yeast and mold counts on commodity surfaces through improved reduction of cross-contamination in wash/flume water systems. Further research is needed to compare the fungicidal efficacy of peroxyacetic/octanoic acid mixtures to other commonly used antimicrobial agents.

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