Efficacy of FIT Produce Wash and Chlorine Dioxide on Pathogen Control in Fresh Potatoes

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ABSTRACT: A commercial fresh pack potato operation was used as a model to evaluate FIT fruit and vegetable wash effectiveness in reducing levels of microorganisms on potatoes and in flume water. Fresh potatoes were washed in flume water with or without FIT, or treated with a spray bar utilizing either FIT, 9 ppm chlorine dioxide (ClO_2), or a water control. Both flume treatments were also evaluated for APC and Gram-negatives. There were no significant differences in reduction of these microorganisms on treated or control potatoes. However, levels of Gram-negative bacteria in FIT-amended flume water were reduced by 5.95 log CFU/g, and the APC was reduced by 1.43 log CFU/g. To validate plant trial findings, this test was repeated using solutions of sterile potato flume water from the fresh pack operation, containing a typical level of dissolved and suspended solids. Treatment solutions prepared with flume water or deionized water containing FIT, 9 ppm ClO_2, or a water control were inoculated with *E. coli* O157:H7, *Salmonella* Typhimurium, or *Pectobacterium carotovorum* ssp. *carotovorum*. FIT and ClO_2 prepared with deionized water reduced levels of microorganisms by >6.1 to 6.6 log CFU/g to below the detection limit. FIT prepared with flume water reduced levels of all organisms by >6.0 to 6.4 log CFU/g to below the detection limit, whereas ClO_2 prepared from flume water reduced bacterial levels of all organisms by only 0.7 to 1.4 log CFU/g. Neither FIT nor ClO_2 was particularly efficacious against *E. coli* O157:H7, *S.* Typhimurium, APC, yeasts, or molds on potato surfaces.

Keywords: FIT, fresh potato processing, potato tuber, produce wash, sanitizer

Introduction

Fresh and ready-to-eat produce has become an increasingly important vehicle for foodborne enteric human pathogens, including *Escherichia coli* O157:H7, *Salmonella* spp., and *Listeria monocytogenes*. Recent outbreaks have included *E. coli* O157:H7 traced to spinach grown in the Salinas Valley of California (CDC 2006) and *S.* Typhimurium associated with consumption of tomatoes (FDA 2006). Washing and sanitizing are an important step in the bacterial control of fresh fruits and vegetables; inadequate sanitation can result in bacterial foodborne illness. Several sanitizers have been examined for their efficacy in killing or reducing pathogenic bacteria such as *E. coli* O157:H7, *Salmonella* spp., and *Listeria monocytogenes* on fresh food products (Beuchat 1999). Washing with tap water cannot completely remove pathogenic bacteria on fresh produce (Brackett 1992). Traditionally, chlorine-based chemicals, widely used for sanitation of fresh whole and cut produce, have been recognized as safe in many countries (Zoffoli and others 1999). Chlorine, in the form of sodium or calcium hypochlorite, applied as a postharvest dip treatment reduced 2 very important postharvest pathogens on apple, sooty blotch (*Gloeodes pomigena* Colby) and flyspeck (*Zygophiala jamaicensis* E. Mason) (Hendrix 1991), as well as fungal rot (Colgan and Johnson 1998) in stored apples and pears. Zoffoli and others (1999) showed that chlorine gas reduced postharvest decay caused by inoculated *Botrytis cinerea* on table grapes (*Vitis vinifera* L.). Postharvest application of chlorine as a spray or dip treatment reduced potato tuber infection by *Alternatia solani* Sorauer (Harrison and Franc 1988) and soft rot caused by *Erwinia carotovora* (Bartz and Kelman 1986). Chlorine dioxide (ClO_2) is a strong sanitizing and oxidizing agent that has a high bactericidal efficacy against foodborne pathogenic bacteria, having approximately 3.5 times the oxidation capacity of chlorine (Benarde and others 1965). Also, aqueous ClO_2 has a bactericidal effectiveness up to 7 times stronger than chlorine when applied to poultry processing chilled water (Lillard 1979). However, production of harmful by-products such as chloramines and trihalomethanes (Cord and Dychadala 1993) as well as greatly reduced bactericidal effectiveness in the presence of soils and organic matter (Zhang and Farber 1996; Lindsay and others 2002; Stampi and others 2002) in wash flumes and dip tanks limits their application as sanitizers to fresh produce. Therefore, alternative sanitizers are needed, which are more effective than chlorine or ClO_2 for suppressing foodborne pathogenic bacteria in fresh produce.

Consumer demand for more "natural," less toxic alternative sanitizers has led industry to develop a number of novel fresh produce sanitizers, including HealthPro Brands Inc.© FIT Fruit and Vegetable Wash™ (Procter & Gamble 2003). In 1 study, FIT was approximately as effective as 200 and 20000 ppm chlorine in reducing populations of *Salmonella* and *E. coli* O157:H7 on alfalfa seeds, but without the human health and environmental hazards associated with such a high level of chlorine (Beuchat and others 2001b). In contrast, the concentration of ClO_2 used in washing fresh produce in commerce is only between 5 and 9 ppm (Personal communication).

This study was undertaken to evaluate liquid and powdered FIT (HealthPro Brands Inc., Cincinnati, Ohio, U.S.A.) in a commercial fresh pack potato operation and in laboratory tests to ascertain its effectiveness against *E. coli* O157:H7, *S.* Typhimurium, *P. carotovorum* ssp. *carotovorum* (vegetable soft rot bacterium), aerobic plate count, yeasts, and molds. Fresh pack potato operations present very challenging conditions for microbial control, due to heavy

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soils on potatoes and in flume water, likely more than with other vegetables.

Materials and Methods

Plant study

Potatoes (cultivar 'Russet Norkotah') from commercial storage were washed for 1 min in a commercial potato flume amended to pH 3.0 to 3.3 with commercial powdered FIT or with a water control. Two potatoes and a corresponding sample of flume water were collected at three 15-min intervals. Both potatoes were shaken and rubbed for 2 min in 50-mL buffered peptone water (BPW, Difco, Chicago, Ill., U.S.A.), tenfold serially diluted in 0.2% peptone water, and 1 mL of appropriate dilutions plated onto aerobic plate count Petrifilm™ and *E. coli*/coliforms Petrifilm™ (3M Microbiology, St. Paul, Minn., U.S.A.), to enumerate Gram-negative bacteria and total mesophilic bacteria, respectively. Plates were incubated for 24 h at 37 °C and for 48 h at 32 °C, respectively, and colonies counted. Samples of flume water were serially diluted in peptone water and processed as described previously. Additional treatments, applied to potatoes on a conveyor belt with a commercial spray bar, included water amended with commercial liquid FIT to pH 3.0, 9 ppm ClO_2 (Adox 750™, Intl. Dioxide Inc., North Kingstown, R.I., U.S.A.), or a water control. The spray bar arrangement consisted of 3 bars with 5 nozzles each, which collectively delivered 30 gallons/min = 113 L/min. Treated potatoes were analyzed as described previously. All tests were replicated on 2 consecutive days.

Cultures and cell suspension

Three nalidixic acid-resistant isolates of *E. coli* O157:H7 (E-2042, E-2026, and E- 2068) and 3 sulfadiazine-resistant isolates of *S.* Typhimurium (S-8983, S-9099, and S-9368) obtained from the Washington State Univ. Veterinary Clinical Sciences Culture Collection were cultured in 9-mL tryptic soy broth without dextrose (TSB, Difco) amended with either 32 ppm nalidixic acid or 100 ppm sulfadiazine for 24 h at 37 °C. The cultures were then centrifuged for 30 min at 2600 × *g*, resuspended to 1/10 the original volume in BPW, yielding cell levels of approximately 1×10^9 CFU/mL, and combined to constitute a mixed culture cocktail. The mixed culture cocktail was stored at 4 °C until use. One isolate of *P. carotovorum* ssp. *carotovorum* (vegetable soft rot bacterium, WSU isolate nr 1) was obtained from the Washington State Univ. Plant Pathology Dept. Culture Collection, cultured for 20 h at 32 °C in 9.0 mL NBY broth [8.0 g nutrient broth (Difco), 2.0 g yeast extract (Difco), 2 g potassium phosphate dibasic, 0.5 g potassium phosphate monobasic, deionized water, 1.0 L; autoclaved for 15 min at 121.1 °C. After being cooled, 5.0 mL of 10% dextrose and 1.0 mL of 1.0 M magnesium sulfate were added]. Cells were harvested by centrifugation at 4000 × *g* for 30 min at 4 °C, and adjusted to approximately 10^9 CFU/mL as described previously.

In vitro tests

Treatment solutions consisted of sterile deionized water mixed with either FIT at the recommended rates (powdered FIT: 5 g/L; liquid FIT: 1 mL/129 mL), 9 ppm ClO_2 (Adox 750), or a deionized water control. One hundred microliters of mixed culture cocktail or *P. carotovorum* ssp. *carotovorum* were added to 9.9 mL of treatment solution. One-milliliter samples were taken at 0-, 0.5-, 1-, 3-, and 5-min intervals, neutralized with 9 mL 1.1 × D/E neutralizing broth (Difco), then appropriate tenfold serial dilutions performed in 0.2% peptone water and spread-plated onto MacConkey-sorbitol agar (SMAC, Difco), xylose lysine desoxycholate agar (XLD, Difco), or NBY agar for enumerating *E. coli* O157:H7, *S.* Typhimurium, or *P. carotovorum* ssp. *carotovorum*, respectively. When low levels of surviving cells were anticipated, 250 μL of the neutralizing D/E broth dilution were spread onto 4 plates for a total of 1 mL. These treatments were repeated using autoclave-sterilized flume water obtained from the fresh pack potato operation, which contained 330 mg/L of total dissolved solids and 2186 mg/L of total suspended solids. Total dissolved solids and total suspended solids were determined using the EPA method (EPA 1999). All treatments were identical to plant trial replicates, except that FIT was added to flume water to pH 3.0. All treatments were replicated 3 times.

Potato tuber tests

Unwashed potato tubers obtained from commercial storage were quartered and allowed to suberize 24 h at room temperature (22 °C). One hundred microliters of enteric pathogen cocktail were applied as 10 droplets onto the uncut surfaces of the potato pieces and dried for 1 to 2 h in a biosafety hood with the fan running. Four potato pieces were submerged in 500 mL treatment solutions prepared from sterile flume water, sampled at 0.5, 1, 3, and 5 min, shaken and rubbed for 2 min in 50 mL D/E broth, then diluted and plated as described previously, except that SMAC was amended with 32 ppm nalidixic acid and XLD with 100 ppm sulfadiazine. Additional inoculated but untreated potato pieces were shaken and rubbed in 50 mL BPW to determine the initial inoculum level ("zero" time). Uninoculated potato pieces were treated as described previously and appropriate dilutions plated on aerobic plate count petrifilm and on potato dextrose agar (PDA, Difco) acidified to pH 3.5 with tartaric acid to enumerate APC, and yeasts and molds, respectively. All tests were replicated 3 times and performed at room temperature (22 ± 2 °C).

Statistical analysis

Three replicate trials for each experiment were performed. The results of microbiological tests were transformed into $\log_{10}$ values and analyzed by analysis of variance (ANOVA) using the procedure of SAS (SAS Inst. Inc., Cary, N.C., U.S.A.). When the effect was significant ($P < 0.05$), means were separated using Duncan's multiple range test.

Results and Discussion

There were no significant differences ($P < 0.05$) in the levels of Gram-negative bacteria or APC on any treated potatoes compared to the controls. However, the levels of Gram-negative bacteria in FIT-amended flume water from plant trials were reduced by 5.95 log CFU/g to below the detection limit, and the APC was reduced by 1.43 log CFU/g (Figure 1 and 2).

In deionized water *in vitro* tests, both formulations of FIT and ClO_2 reduced the levels of all tested bacteria to below the detection limit within 30 s. Log reductions were > 6.43 (*E. coli* O157:H7), >6.65 (*S.* Typhimurium), and > 6.14 (*P. carotovorum* ssp. *carotovorum*). This pattern was also observed for sterile flume water treated with FIT (Figure 3 to 5). Log reductions after 30-s treatment were >6.43 (*E. coli* O157:H7) > 6.50 (*S.* Typhimurium), and > 6.11 (*P. carotovorum* ssp. *carotovorum*). However, flume water amended with ClO_2 only reduced bacteria after 5 min by 1.03 log (*E. coli* O157:H7), 1.17 log (*S.* Typhimurium), and 1.40 log CFU/g (*P. carotovorum* ssp. *carotovorum*).

The effectiveness of sanitizers in reducing bacteria on potato tubers was greatly curtailed compared to the reduction that occurred in inoculated water (Figure 6 to 10). After 5 min, flume water amended with powdered FIT, liquid FIT, and ClO_2 reduced levels

of *E. coli* O157:H7 by 1.44, 1.52, and 0.89 log CFU/g, respectively. *S.* Typhimurium was reduced by 1.36, 1.83, and 0.86 log CFU/g, respectively. The flume water control reduced *E. coli* O157:H7 and *S.* Typhimurium on inoculated tubers by 0.71 and 0.78 log CFU/g, respectively. After 5 min, powdered FIT, liquid FIT, and ClO_2 treatments reduced the APC by 1.03, 1.20, and 0.33 log CFU/g, respectively. Flume water alone produced a 0.42 log CFU/g reduction. Yeast microflora was reduced by 0.41, 1.20, and 0.16 log CFU/g, respectively, compared to the flume water control, which reduced yeasts by 0.87 log. Mold microflora was reduced by 0.90, 0.85, and

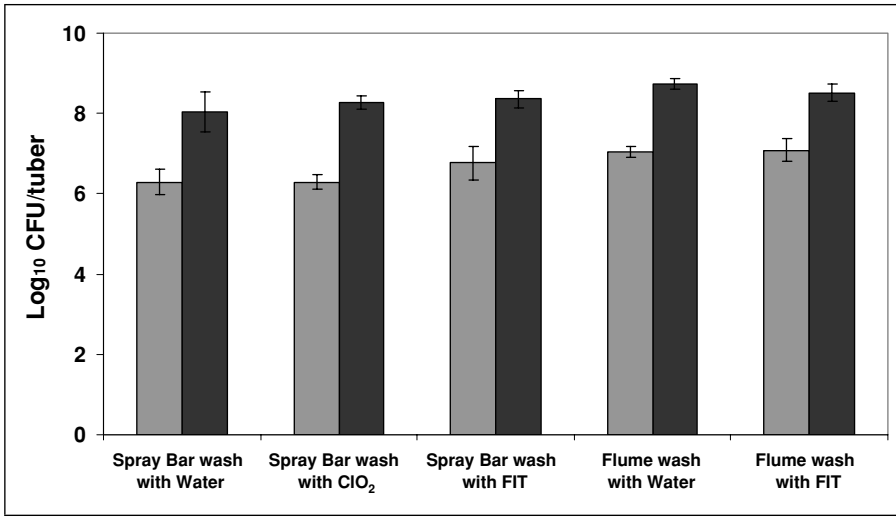

Figure 1—Efficacy of FIT, ClO_2, and water control treatment with a commercial spray bar, and flume treatment in reducing levels of Gram-negative bacteria and aerobic plate count (APC) on potato tuber surfaces. ■, Gram-negative bacteria; ■, aerobic plate count.

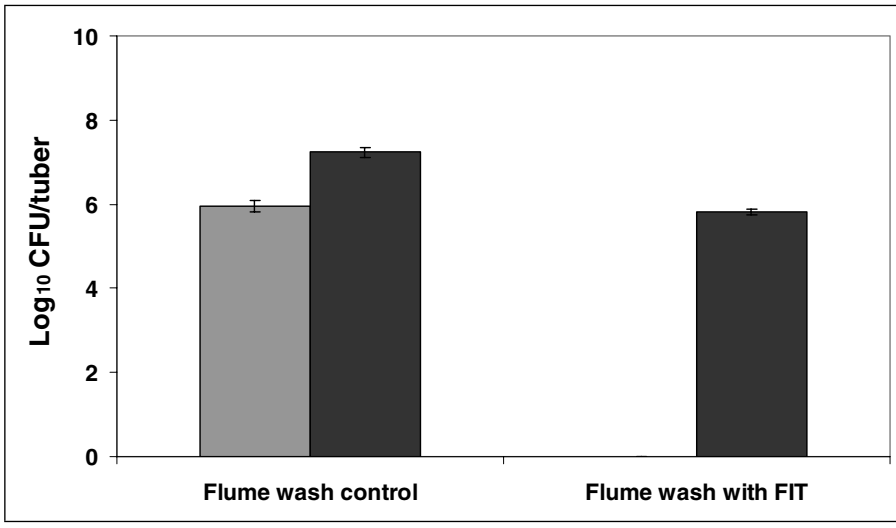

Figure 2—Efficacy of FIT and water control treatment in reducing levels of Gram-negative bacteria and aerobic plate count (APC) in a potato flume. ■, Gram-negative bacteria; ■, aerobic plate count.

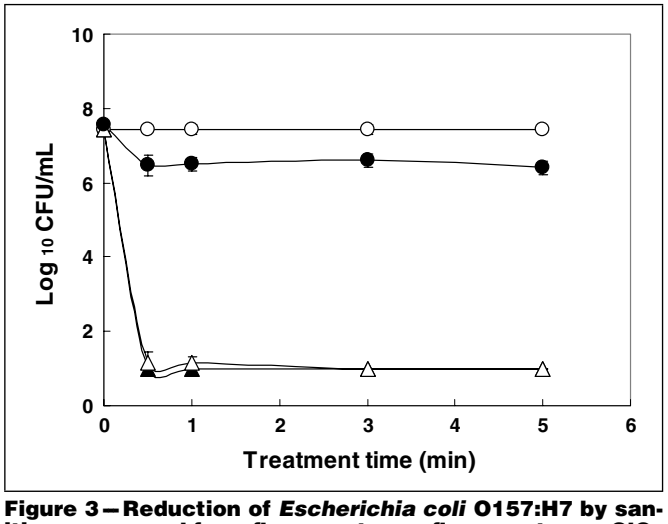

Figure 3—Reduction of *Escherichia coli* O157:H7 by sanitizers prepared from flume water. ○, flume water; ●, ClO_2 9 ppm; △, liquid FIT pH 3.0; ▲, dry FIT pH 3.0.

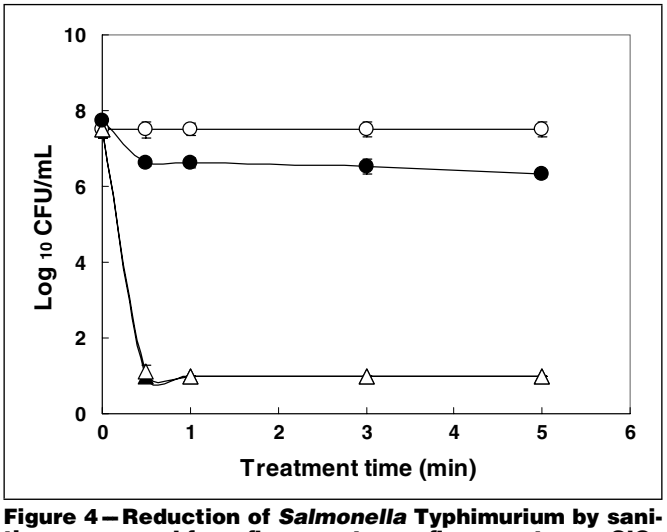

Figure 4—Reduction of *Salmonella* Typhimurium by sanitizers prepared from flume water. ○, flume water; ●, ClO_2 9 ppm; △, liquid FIT pH 3.0; ▲, dry FIT pH 3.0.

0.05 log CFU/g, respectively. The flume water control, reduced molds by 0.79 log CFU/g after 5 min.

All tested enteric pathogen strains were adapted to grow in the presence of 32 ppm nalidixic acid or 100 ppm sulfadiazine to suppress interfering Gram-negative bacteria originating from fresh produce (Beuchat and others 2001a). *E. coli* O157:H7, *S.* Typhimurium, and *P. carotovorum* were chosen for the efficacy study of FIT. *E. coli* O157:H7 and *S.* Typhimurium have been implicated in outbreaks associated with fresh produce (FDA 2006). Also, *Pectobacterium carotovorum* (syn. *Erwinia carotovora*) is a phytopathogen and has been isolated from various crops, including potatoes, tomatoes, cabbages, carrots, cucumber, and maize (Dye 1969; Perombelon and Kelman 1980). FIT fruit and vegetable wash was highly effective at rapidly killing both enteric pathogens and *P. carotovorum* ssp. *carotovorum* cell suspensions. Chlorine dioxide (ClO_2), while highly effective when prepared with deionized water, was largely ineffective against bacteria when used to treat flume water. This effect was likely due to suspended and dissolved solids and potato debris. The presence of organic materials reduces the efficacy of chlorinated water (Oomori and others 2000). Beuchat and others (2001b) reported that the presence of organic material in the inoculum influences the efficacy of sanitizers such as chlorine, ClO_2, and FIT produce wash. Conversely, FIT did not lose any effectiveness against bacterial suspensions regardless of water quality. However, all sanitizers were much less effective when applied to potato tubers. Both formulations of FIT in flume water reduced levels of enteric pathogens on potato tuber surfaces by 1.4 to 1.8 log CFU/g after 5 min, whereas ClO_2 was no more effective than the flume water control (< 0.9 log reduction). Bartz and Kelman (1986) discovered that chemical treatments and drying were less effective on potato tubers that had been contaminated with *E. carotovora* (soft rot bacteria) or had numerous injuries. Lenticels and other potato tuber surface features may offer protection against sanitizers.

FIT produce wash was more effective than 20000 ppm of chlorine in killing *E. coli* O157:H7 and *Salmonella* spp. on alfalfa seeds (Beuchat and others 2001b). Beuchat and others (2001a) reported that treatment with 200 ppm chlorine and FIT showed ≥ 3.07 and >6.83 $\log_{10}$ reductions of *Salmonella* spp. on tomatoes,

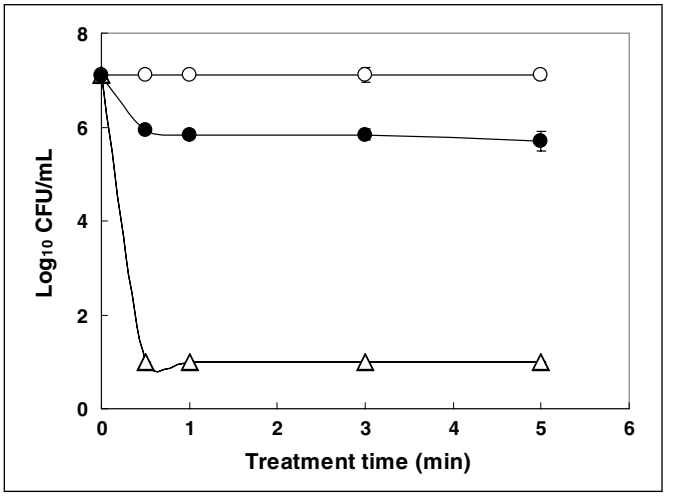

Figure 5—Reduction of *Pectobacterium carotovorum* ssp. *carotovorum* by sanitizers prepared from flume water. ○, flume water; ●, ClO_2 9 ppm; △, liquid FIT pH 3.0; ▲, dry FIT pH 3.0.

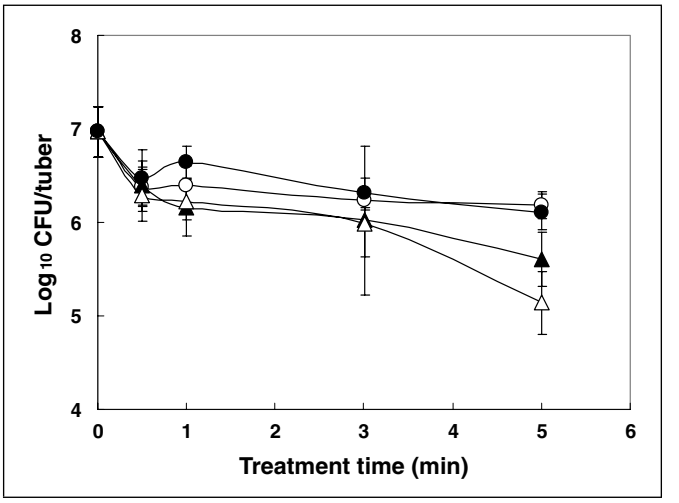

Figure 7—Reduction of *Salmonella* Typhimurium on inoculated potato tubers by sanitizers. ○, flume water; ●, ClO_2 9 ppm; △, liquid FIT pH 3.0; ▲, dry FIT pH 3.0.

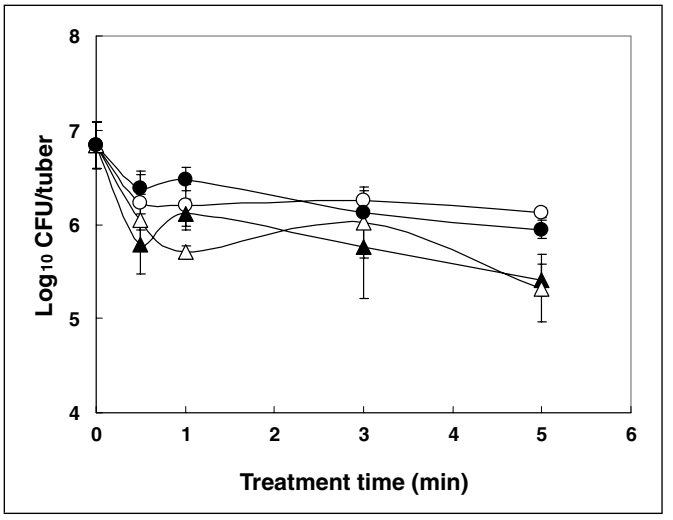

Figure 6—Reduction of *Escherichia coli* O157:H7 on inoculated potato tubers by sanitizers. ○, flume water; ●, ClO_2 9 ppm; △, liquid FIT pH 3.0; ▲, dry FIT pH 3.0.

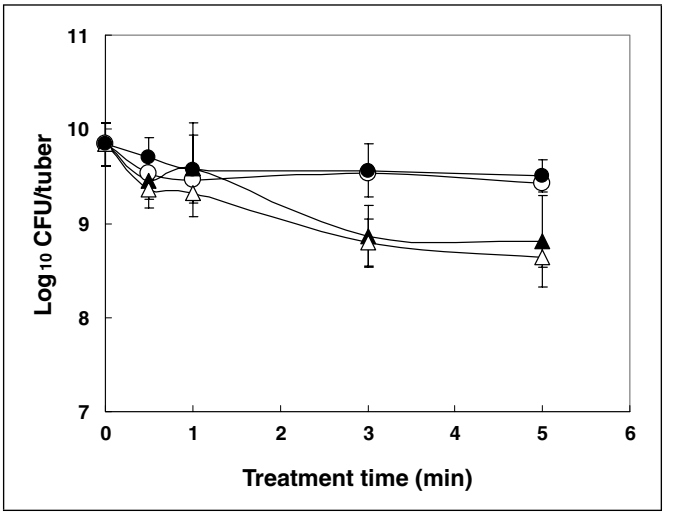

Figure 8—Reduction of aerobic plate count (APC) on potato tubers by sanitizers. ○, flume water; ●, ClO_2 9 ppm; △, liquid FIT pH 3.0; ▲, dry FIT pH 3.0.

respectively. FIT produce wash was demonstrated to have bactericidal activity against foodborne pathogens that exceeded the activity of either 20000 or 200 ppm chlorine. Taormina and Beuchat (1999) examined the residual concentration of free chlorine in solutions within 15 min. In their study, free chlorine in 200 and 20000 ppm solution decreased by approximately 20 and 16000 ppm, respectively. However, FIT produce wash, due to its antimicrobial activity in water with high levels of dissolved and suspended solids, would be expected to result in greatly reduced cross-contamination of produce coming out of a flume. Moreover, FIT produce wash consists of GRAS (generally recognized as safe) ingredients (water, oleic acid, glycerol, ethanol, potassium hydroxide, citric acid, and distilled grapefruit oil) (Beuchat and others 2001a). Thus, the use of FIT produce wash to sanitize fresh pack potatoes would greatly reduce hazards to workers and to the environment, compared to chlorinated compounds.

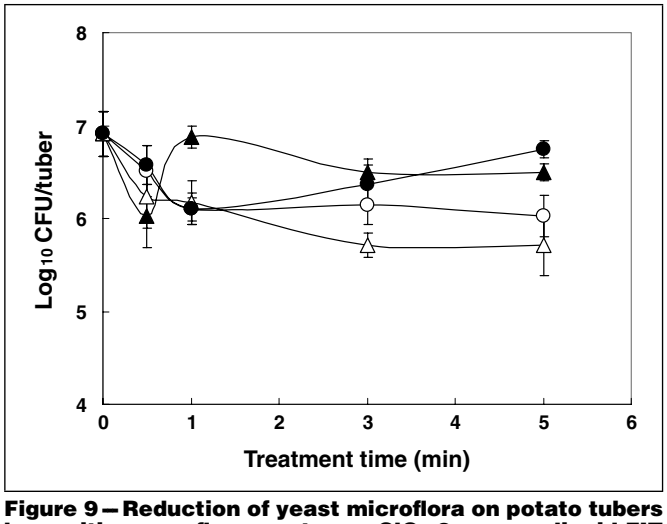

Figure 9—Reduction of yeast microflora on potato tubers by sanitizers. ○, flume water; ●, ClO_2 9 ppm; △, liquid FIT pH 3.0; ▲, dry FIT pH 3.0.

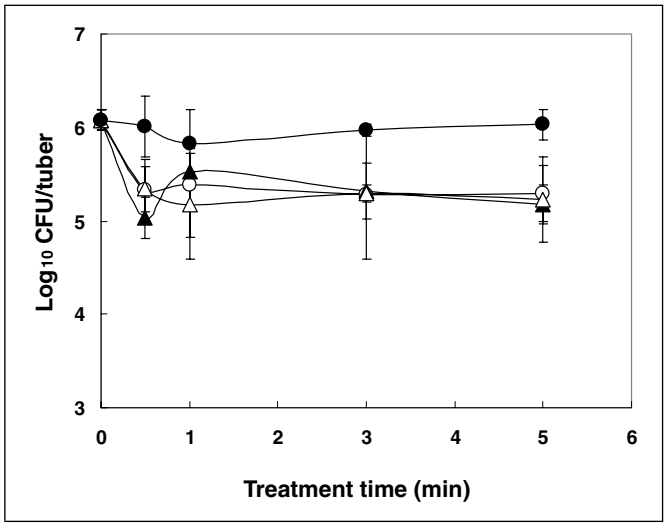

Figure 10—Reduction of mold microflora on potato tubers by sanitizers. ○, flume water; ●, ClO_2 9 ppm; △, liquid FIT pH 3.0; ▲, dry FIT pH 3.0.

Conclusions

This study investigated the potential efficacy of FIT produce wash in a commercial fresh pack potato operation. FIT showed significantly greater reductions in populations of *E. coli*, *S.* Typhimurium, and *P. carotovorum* ssp. *carotovorum* than treatments consisting of water or 9 ppm ClO_2. Similarly, there were similar and significant, though lower, reductions in populations of *E. coli*, *S.* Typhimurium, APC, yeast, and mold in potato tubers. The results indicate that FIT showed significant potential for preventing cross-contamination with bacterial pathogens in vegetable flumes and dip tanks. FIT produce wash may prove to be a more convenient and safer alternative to chlorinated sanitizers in food processing applications. Further research utilizing different vegetable models should be pursued.

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