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PEER-REVIEWED ARTICLE

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Efficacy of Peracetic Acid–based Sprays against Microbial Loads on Conveyors in a Walnut Hulling Facility

ABSTRACT

Association of foodborne pathogens with tree nuts has led to interest in microbial mitigation strategies that can be applied during postharvest handling and processing. The efficacy of commercial peracetic acid (PAA)-based sanitizer spray applications was evaluated for efficacy in reducing aerobic plate counts (APC) and *Escherichia coli*/coliform counts (ECC) on conveyor belts in a commercial walnut huller. Water alone was compared to one of four PAA-based sanitizers at concentrations of 100 or 200 ppm PAA. APC and ECC were significantly ($P < 0.05$) lower on conveyor belts sprayed with 200 ppm PAA than on those sprayed with water. Significantly ($P < 0.05$) lower APC and ECC were observed on conveyor belts sprayed with one PAA formulation at 100 ppm (5.00 and 4.14 log CFU/100 cm^2, respectively) than on those sprayed with water (6.40 and 6.10 log CFU/100 cm^2, respectively). The efficacy of this sanitizer was not significantly different ($P > 0.05$) at 25, 50, 80, or 100 ppm (APC: 4.32 to 4.51 log CFU/100 cm^2; ECC: 2.79 to 2.87 log CFU/100 cm^2). PAA sprays reduce microbial levels on conveyer belt surfaces in walnut hulling facilities, which may reduce the potential for cross-contamination.

INTRODUCTION

Since 2001, tree nuts, peanuts, and seeds have been associated with several outbreaks of foodborne illness *(17)*. Shelled walnuts were epidemiologically linked to an outbreak of *Escherichia coli* O157:H7 gastroenteritis in Canada *(7)*. Although the organism was not recovered from the outbreak-implicated walnuts, there have been several separate recalls of walnut kernels in the United States initiated after in-product detection of *Salmonella (19, 20, 23–25)*, *E. coli* O157:H7 *(7, 8)*, and *Listeria monocytogenes (18, 21, 22)*. The prevalence of *Salmonella* in 2,903 California-grown inshell walnuts collected shortly after harvest, hulling, and drying was 0.14% (375 g; 95% confidence interval [CI], 0.054 to 0.35%), and no *E. coli* O157:H7 was recovered from any of the same

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samples (<0.034% prevalence; 95% CI, 0 to 0.13%) *(10)*. *Salmonella* and *E. coli* O157:H7 could be recovered from inoculated inshell walnuts and walnut kernels during several months of ambient storage even at initially low inoculum levels *(2, 4, 15)*. Increased survival was observed at cooler storage temperatures.

The contamination source has not been identified when pathogens have been isolated from walnuts. Like most foods, walnuts have the potential to become contaminated at any point in the supply chain. At harvest, walnuts are mechanically shaken from the trees, collected from the orchard floor by mechanical sweeping, and then transported to a huller-dehydrator. The capacity and layout of huller-dehydrators vary, but all are designed to separate the walnuts from debris, to remove hulls that may be adhering to the inshell nuts, and to dry the walnuts to <8% moisture *(16)*. For one of the initial separation steps, huller-dehydrators typically employ a tank of water, referred to as a float tank, to separate the walnuts (which float) from rocks (which sink) that are inadvertently collected from the orchard.

Aerobic plate counts of ≥6 log CFU/ml have been measured in float tank water *(1)*, increasing the potential for distribution of contaminants within and among loads of walnuts. Microbial loads (aerobic plate counts [APC] and *E. coli*/coliform counts [ECC]) measured on inshell walnuts collected from the tree were 6 and 4 log CFU/nut, respectively *(16)*. These levels increased by ~1 log during the hulling process, decreased by ~1 log during drying, and decreased by a further ~2 logs in the initial months of commercial storage *(15)*; microbial levels were often significantly higher on kernels extracted from walnuts with visibly broken shells *(16)*.

Sanitizers are not typically added to the float tank water because of the presence of very high levels of organic matter *(11, 12)* and the sensitivity of walnut kernels to oxidizing agents. Addition of 25 ppm peracetic acid (PAA)-based sanitizer to the float tank water failed to significantly reduce microbial populations on walnuts under commercial conditions *(11)*. Contact times were very short (seconds), and maintaining target sanitizer levels was a challenge. Despite the negligible impact on microbial loads, sensory analysis by a trained panel indicated that walnut quality was negatively impacted by inclusion of PAA in the float tank.

Frelka and Harris *(16)* evaluated the effect of a water or PAA spray applied to walnuts immediately after hulling. Although consistent sanitizer concentration could be maintained using this approach, the application times were short (~13 s). In some cases, reductions of APC and ECC were significantly ($P < 0.05$) greater on PAA-sprayed (100 or 200 ppm) freshly-hulled walnuts than on water-sprayed walnuts. However, average reductions were <1 log CFU/nut in either case. The low concentrations, short contact times, and irregular surface features of the walnut shell were thought to impact sanitizer performance.

The objective of the current study was to evaluate the efficacy of several PAA-based spray applications for reducing APC and ECC (as indicators of sanitation) on conveyor belts in a commercial walnut huller. It was hypothesized that the PAA sprays would significantly reduce APC and ECC when applied to these conveyors, because the belt surface is smooth and sustained exposure times are possible.

MATERIALS AND METHODS

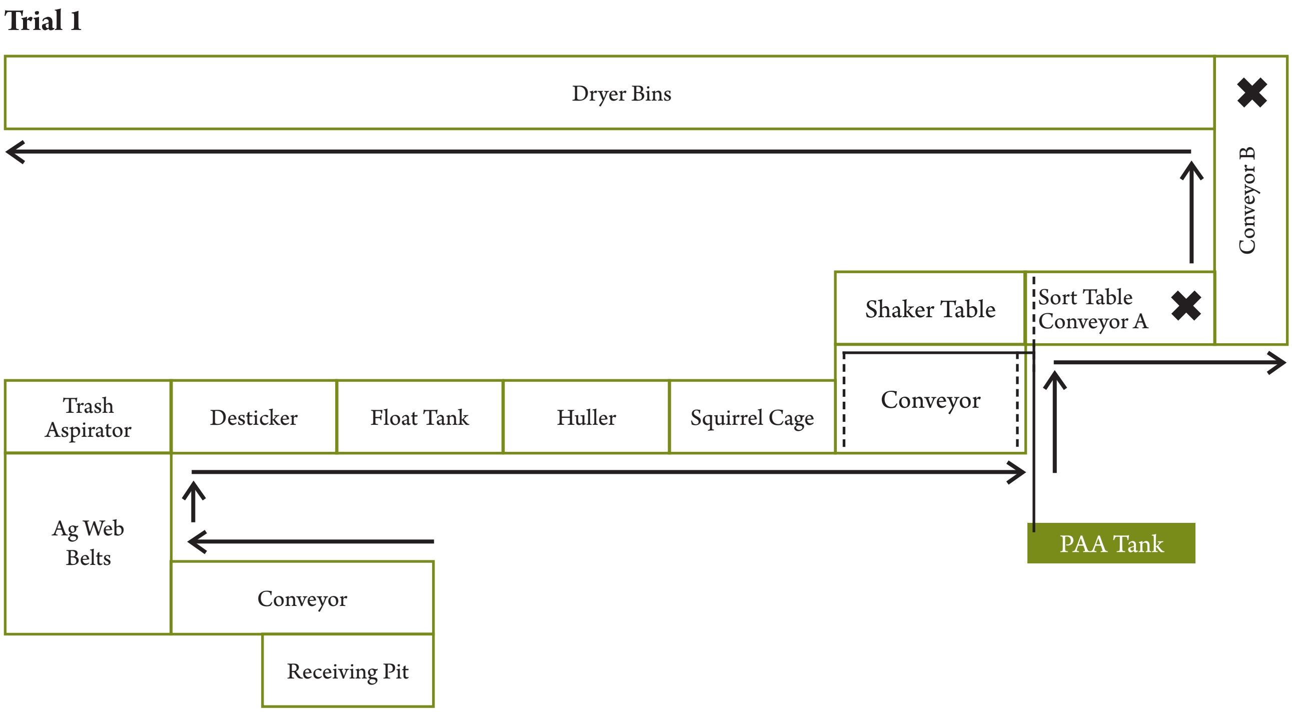
Cooperating facility

During two harvest years (2012 and 2014; trials 1 and 2, respectively), the efficacies of peracetic acid-based sanitizing agents in reducing APC and ECC on smooth-surface conveyor belts in a commercial walnut huller-dehydrator were evaluated under otherwise typical operational conditions. The huller-dehydrator facility, located near Stockton, California, was a full-scale operation capable of processing approximately 45,000 kg/h under peak conditions. The facility layout was typical for the walnut industry *(Fig. 1)*. At the end of each day after hulling, the float tank was emptied; the float tank and hulling equipment were sprayed with water to remove visible debris and then allowed to dry overnight. The float tank was refilled with approximately 1,500 liters of well water the following morning before hulling began.

Antimicrobial treatments

Four different commercial peracetic acid (PAA) formulations, applied at concentrations of 100 and/or 200 ppm, were evaluated during trial 1; each product differed in the relative concentrations of PAA and hydrogen peroxide (H_2O_2) *(Table 1)*. A single formulation (SaniDate 5.0, BioSafe Systems, East Hartford, CT), selected for further examination in trial 2, was applied at concentrations of 25, 50, 80, and 100 ppm. A spray system for the antimicrobial treatments was installed in the hulling facility, as described previously *(16)*. The products were applied to the freshly hulled inshell walnuts and the conveyor below them with spray nozzles (46500A-1-PP-VI, ProMax Clip Eyelet with QPTA6505 ProMax Quick VeeJet nozzles; Spraying Systems Co., Wheaton, IL) at a rate of 1.9 liters/min/nozzle. In trial 1, 18 overhead nozzles (total flow rate = 34.2 liters/min) were used: 13 nozzles were distributed over a 2-m mesh belt, which was followed by a shaker table leading to a 1-m mesh belt with five overhead nozzles *(Fig. 1)*. In trial 2, 19 nozzles (total flow rate = 35 liters/min) were used: the shaker-table nozzles were increased from five to six and relocated to above the front end of conveyor B *(Fig. 1)*. The PAA dosage was controlled by a ProMinent Dulcometer diaLog DACa controller (ProMinent Fluid Controls, Inc., Pittsburgh, PA).

On each sampling day, potable well water was applied through the spray system before any antimicrobial was added. The water was sampled at the spray nozzle and APC and ECC were measured as described later. Hulling equipment ran at



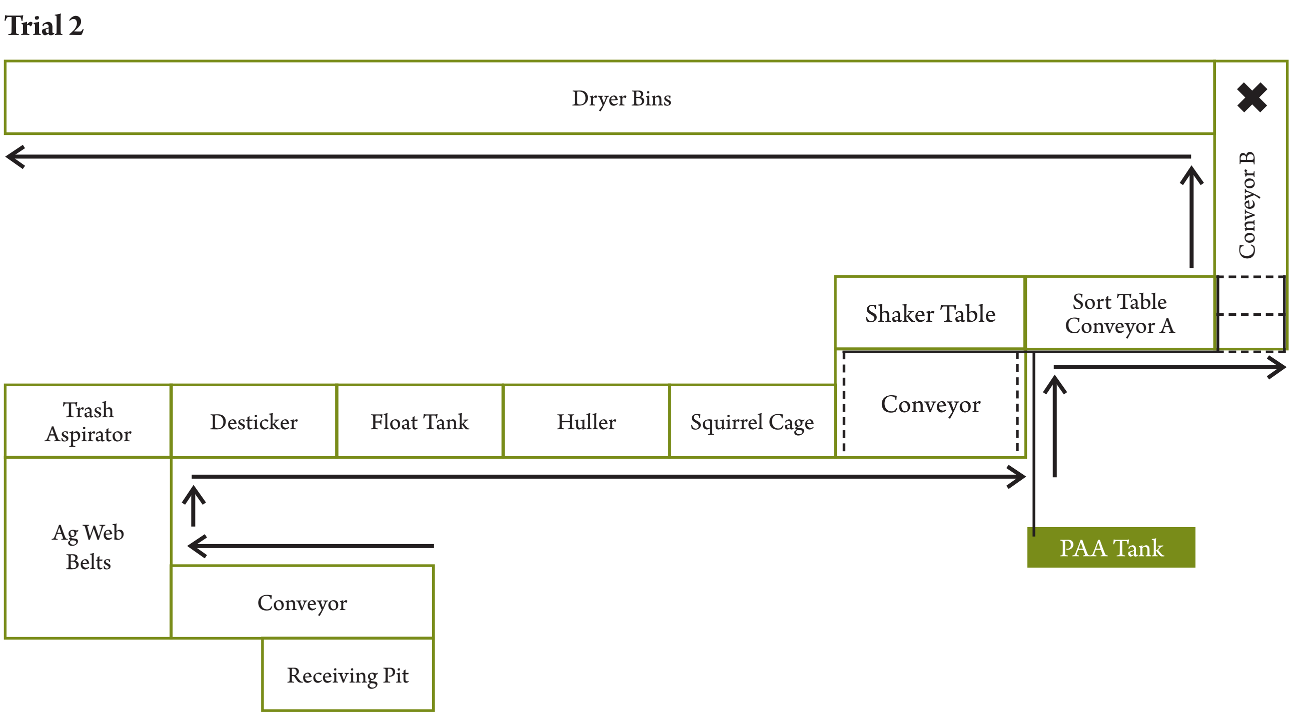


Figure 1. Diagram of the walnut huller-dehydrator used in trial 1 and trial 2. The sampling locations are indicated by the "X". The peracetic acid (PAA) tank is shown in green and the spray line locations are shown with dashed lines. The direction of flow through the system is indicated by the arrows.

TABLE 1. Summary of antimicrobial products tested

Product	Manufacturer	Active ingredient conc. (% by wt)	
		PAA[a]	H_2O_2[b]
StorOx 2.0	BioSafe Systems Co. (East Hartford, CT)	2.0	27.0
SaniDate 5.0	BioSafe Systems Co.	5.3	23.0
SaniDate 12.0	BioSafe Systems Co.	12.0	18.5
BioSide HS 15%	EnviroTech (Modesto, CA)	15.0	22.0

[a] PAA, peracetic acid.
[b] H_2O_2, hydrogen peroxide.

normal speeds with walnuts for at least 30 min before control (water sprayed) samples were collected. After switching, antimicrobial sprays were applied for at least 15 min (to allow systems to become saturated with the solutions) before test samples were collected.

Sampling of float tank water

Samples were collected with sterile 250-ml water samplers (Sterilin, Stafford, UK) at the beginning of the day at the time of equipment startup, at 30 min, and then at 1, 2, and 3 h. Water samples were held on ice no more than 2 h before analysis.

Sampling of conveyor belts

In trial 1, two separate smooth-surface polyvinyl chloride belt conveyors (ContiTech North America Inc., Montvale, NJ) were sampled to evaluate the microbial loads on the sort table conveyor (conveyor A) and on a cross-conveyor leading from the sort table to the dryer bins (conveyor B) *(Fig. 1)*. In trial 2, only conveyor B was sampled because of the reconfiguration of the sprayers *(Fig. 1)*. Samples were collected when the facility was in operation and while either a water (control) or antimicrobial spray was being applied to the conveyors. The conveyors were sampled by lightly pressing a sterile cellulose sponge pre-moistened with 10 ml of Dey-Engley neutralizing broth (Solar Biologicals Inc., Ogdensburg, NY) to the moving belt for 5 s. The DE was used to inactivate any residual test antimicrobials. The belt speeds, as determined with a tachymeter, were used to calculate the area of conveyor belt sampled. All samples were quickly placed on ice and then processed within 1 h in an off-site laboratory located approximately 8 km from the huller-dehydrator facility.

Preparation of samples for analysis

Each sponge used to sample the conveyors was placed into a separate 530-ml (18-oz) Whirl-Pak bag (Nasco, Modesto, CA) with 20 ml of Dey-Engley (DE) neutralizing broth (Difco brand, BD, Franklin Lakes, NJ), and the samples were homogenized for 30 s at high speed in a Smasher blender-homogenizer (AES Chemunex, Combourg, France).

Microbial analysis

Serial 10-fold dilutions of the prepared samples were made in 9 ml of Butterfield's phosphate buffer (Hardy Diagnostics, Santa Maria, CA). Appropriate dilutions were plated onto tryptic soy agar (TSA; Difco brand, BD) supplemented with cycloheximide (cyclo) (Acros Organics, Geel, Belgium) at 50 mg/liter to reduce mold growth and onto CHROMagar ECC plates (CHROMagar, Paris, France) and incubated at 37°C for 24 h. After 24 h, all colonies visible on TSA were included in the APC count, and all pink (coliform) and blue (presumptive *E. coli*) colonies on CHROMagar ECC were enumerated; results were reported as CFU/100 cm^2.

Statistical analysis

In most cases, the antimicrobial treatments were evaluated over two applications that occurred on separate days; three single-sponge samples per location were collected during each antimicrobial application (n = 6). In trial 1, the treatment with BioSide HS 15% was applied twice on the same day, with a 3-h gap between samplings; three single-sponge samples were collected after a single treatment with BioSide HS 15% at 100 and 200 ppm (n = 3). Reductions were calculated by averaging the calculated differences between the average initial log CFU/100 cm^2 and the individual log CFU/100 cm^2 measured for each of six water or PAA-sprayed samples. Statistical analyses, including analysis of variance and Tukey-Kramer HSD tests, were performed with JMP 11 software (SAS Institute, Cary, NC). Differences between means were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

The United States is one of the top producers of walnuts in the world; more than 4,000 growers in California produce over 99% of the walnuts grown in the United States *(5)*.

Currently, there are approximately 400 huller-dehydrator facilities in the state, which range widely in their size and sophistication. The process is typically considered an extension of on-farm activities, with a goal of removing the hull and reducing the moisture to stabilize the product. After drying, hulled walnuts are transported to one of the ~90 handlers and/or processing facilities in California, where the nuts are stored in bins or silos until they are further processed and distributed. Small huller-dehydrators may handle several thousand kilograms of product from a single grower, whereas a larger operation may provide this service to a wide range of growers and handle thousands of metric tons of product through a typical harvest.

Changes in microbial loads on conveyer belts during initial operation

Walnuts are passed through a tank of water immediately before hulling, which allows rocks to sink and thus reduces the risk for damage to the hulling equipment. In a preliminary study, baseline microbial loads were determined for the float tank water and conveyor belts at the beginning of the day before any walnuts were hulled and then during the first 3 h of hulling. The equipment was operated at standard speed with walnuts that were sprayed with water (2.08 and 1.12 log CFU/ml for APC and ECC, respectively) after hulling. Initial float tank water APC and ECC of 6.51 ± 0.12 and 5.99 ± 0.09 log CFU/ml, respectively, increased to 7.23 ± 0.04 and 6.98 ± 0.07 log CFU/ml, respectively, after the first hour *(Fig. 2)*. The maximum APC and ECC, 7.33 ± 0.10 and 7.17 ± 0.04 log CFU/ml, respectively, were measured at 3 h. These data are consistent with a previous study, in which APC of >6 log CFU/ml and *Enterobacteriaceae* counts of ~6 log CFU/ml were measured in float tank water collected from two huller-dehydrators *(1)*.

After the float tank was emptied at the end of the day, the empty tank and surrounding equipment were rinsed with potable well water and allowed to dry. This practice reduced but did not eliminate hull debris, likely contributing to the initial microbial levels observed when the tank was refilled with potable well water at the start of the next day. Regardless of the initial microbial levels in the float tank, when the in-hull walnuts (along with orchard debris and soil) are added to the float tank, they immediately introduce a rich source of microorganisms *(3)*. APC and ECC on walnut hulls significantly increase as walnuts mature, and APC can increase in harvested, moist, brown hull material *(3)*.

Conveyor systems transport hulled walnuts from the huller to the dehydrator bins. The initial APC was 2.80 ± 0.55 and 4.13 ± 1.22 log CFU/100 cm^2 for conveyer belts A and B, respectively, before hulling began; these levels increased significantly ($P < 0.05$) to 6.79 ± 0.07 and 6.53 ± 0.07 log CFU/100 cm^2, respectively, after hulling walnuts for 30 min *(Fig. 2)*. Similar significant increases were observed for the ECC; initial levels of 1.93 ± 0.58 and 3.93 ± 1.13 log CFU/100 cm^2, respectively, increased to 6.12 ± 0.21 and 6.27 ± 0.21 log CFU/100 cm^2, respectively, for belts A and B in the first 30 min of operation. From 0.5 to 3 h, the microbial loads on the conveyor belts fluctuated between 5.73 ± 0.65 and 6.79 ± 0.07 log CFU/100 cm^2 and between 5.02 ± 0.53 and 6.27 ± 0.21 CFU/100 cm^2 for APC and ECC, respectively.

PAA is a common sanitizer option for maintaining water quality in produce water flumes or in recirculating water systems and is considered particularly useful where higher organic loads are anticipated *(9)*. PAA-based sanitizers are mixtures of H_2O_2, acetic acid, and PAA in equilibrium, and may contain chemical stabilizers. PAA also is recognized as a food processing equipment sanitizer and has been shown to be effective against foodborne pathogens on food contact surfaces, including conveyor systems *(26)*, and in the presence of organic loads or in the presence of a biofilm *(14)*. The breakdown products of PAA (acetic acid and water) are nonhazardous, which reduces issues with disposal of wastewater. In previous studies, neither the addition of 25 ppm PAA to float tank water nor the spray application of PAA to walnuts consistently and significantly reduced microbial loads on the surface of walnuts *(11, 16)*. However, it was hypothesized that PAA might be useful as a sanitizer when applied directly to huller-dehydrator conveyor belts, given the relatively smooth surfaces, the potential for continual application, and the sanitizer's demonstrated effectiveness in high organic load environments.

Impact of antimicrobial sprays on microbial loads of conveyer belts

In trial 1, four different formulations and two concentrations of PAA-based antimicrobial sprays were evaluated for efficacy in reducing microbial loads on huller conveyer belts. The application of the sanitizing agents to the conveyor belts in trial 1 was indirect, because the sprays were intentionally applied to the walnuts and then carried to conveyor belt A and B surfaces via the walnuts. Compared with results with the water spray, significantly ($P < 0.05$) lower APC and ECC were observed on conveyer belts A and B for all but two treatments (BioSide HS 15% and SaniDate 12.0, 100 ppm) *(Fig. 3)*. Average reductions for all PAA sanitizers were similar ($P > 0.05$) for both conveyor belts A (1.16 ± 0.89 log CFU/100 cm^2 [APC] and 2.17 ± 1.24 log CFU/100 cm^2 [ECC]) and B (1.26 ± 0.87 log CFU/100 cm^2 [APC] and 2.32 ± 1.27 log CFU/100 cm^2 [ECC]). With 200 ppm SaniDate 5.0, the difference in counts between water and the PAA treatment was as much as 2.70 (APC) and 4.27 (ECC) log CFU/100 cm^2. Greater reductions were observed at 200 ppm than at 100 ppm for all products, though the difference was not always significant. During application of PAA sprays, a fine mist of sanitizer was created, which caused an irritation hazard for the employees working in close proximity to the spray bars *(16)*. Respiratory tract irritation was observed even with the lowest tested concentration (100 ppm).

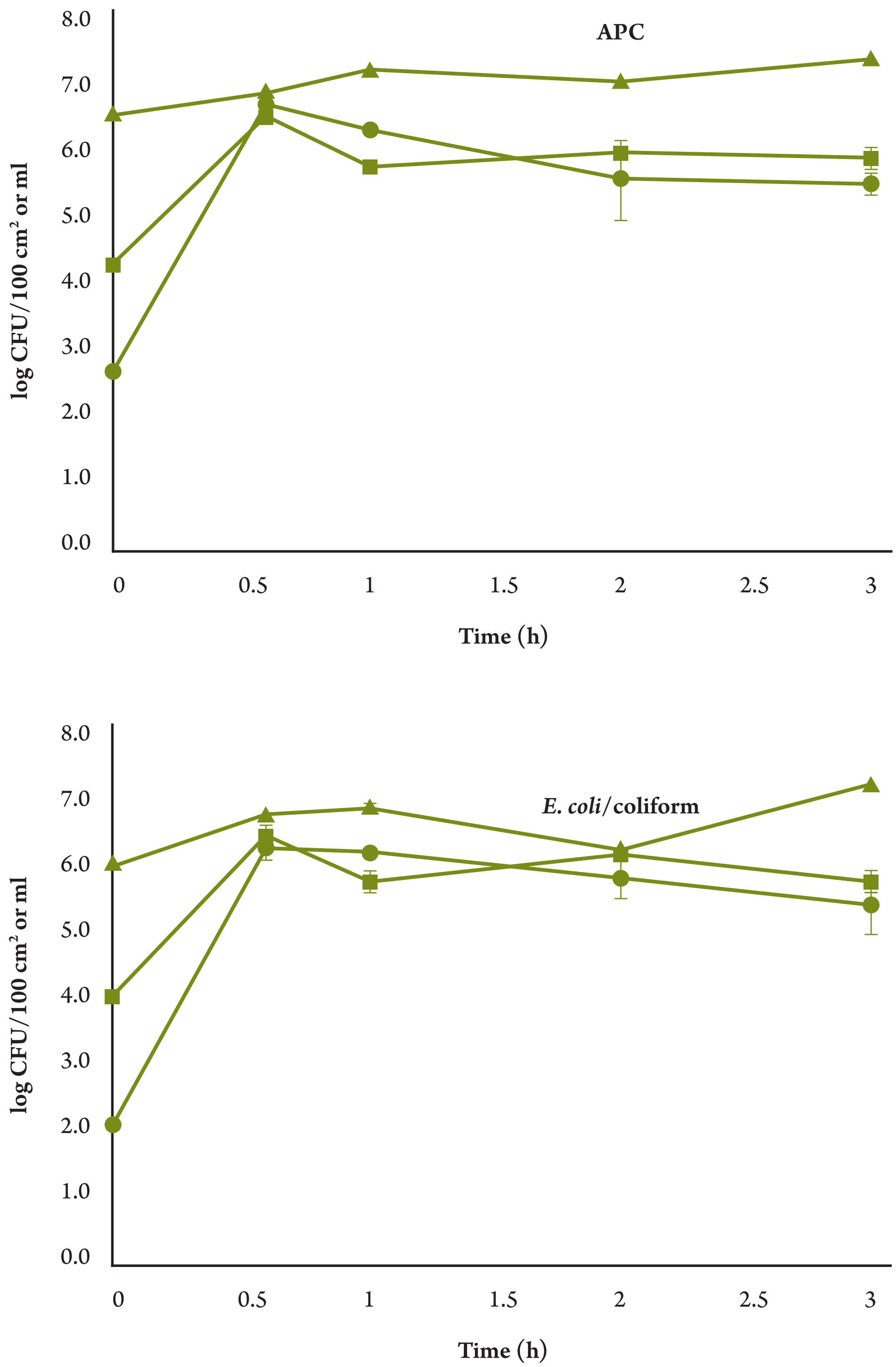


Figure 2. Mean (± SD) aerobic plate counts (APC) and E. coli*/coliform counts from conveyor belt A (circles), conveyor belt B (squares), and float tank water (triangles) sampled from time of equipment startup (n = 3). Water was applied to the walnuts during the 3-h sampling period.*

SaniDate 5.0 was selected for further evaluation in trial 2 because it was the most effective sanitizer in trial 1. Lower PAA concentrations of 25, 50, and 80 ppm, in addition to the previously assessed 100 ppm, were selected in order to examine the efficacy of lower concentrations, with the goal of potentially reducing operational costs, likelihood of equipment corrosion, and respiratory tract irritation of workers. Also, the spray bar was relocated to further

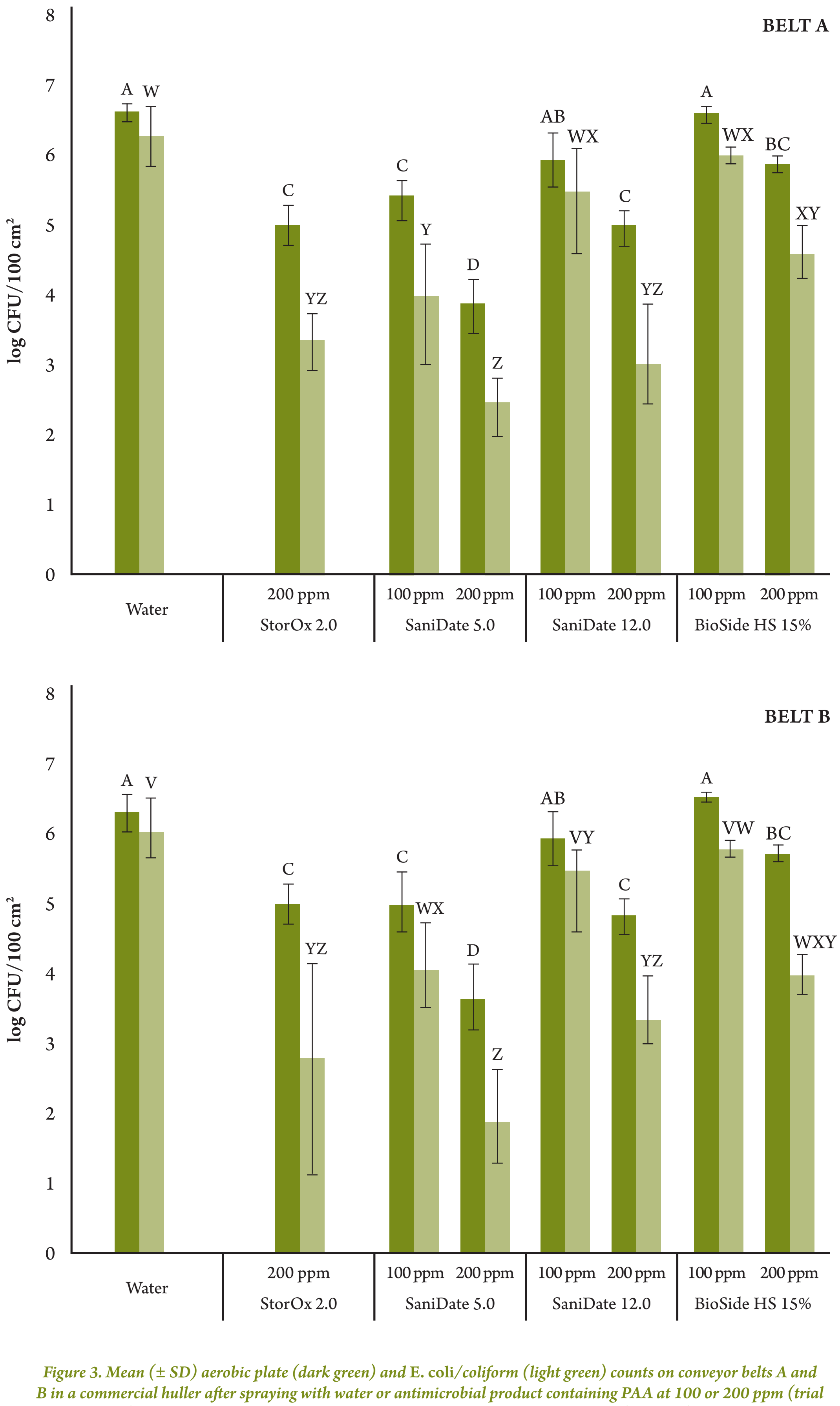


Figure 3. Mean (± SD) aerobic plate (dark green) and E. coli*/coliform (light green) counts on conveyor belts A and B in a commercial huller after spraying with water or antimicrobial product containing PAA at 100 or 200 ppm (trial 1). For APC or ECC, means with different letters are significantly different (P < 0.05); n = 6.*

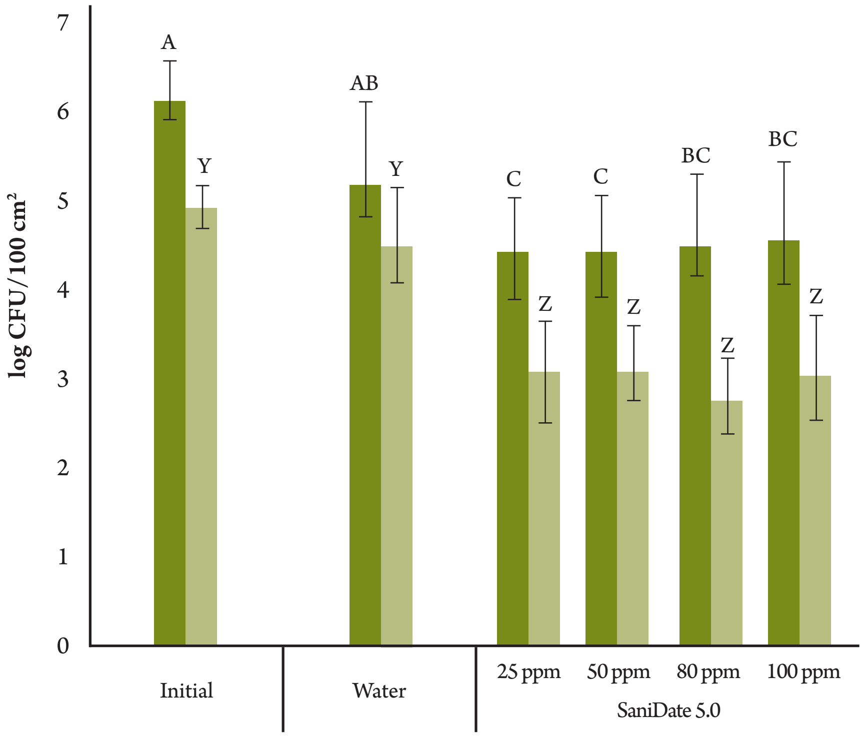


Figure 4. Mean (± SD) aerobic plate (dark green) and E. coli*/coliform (light green) counts on conveyor belt B in a commercial huller collected initially, and after spraying with water or 25, 50, 80, and 100 ppm PAA (SaniDate 5.0) (trial 2). For APC or ECC, means with different letters are significantly different (P < 0.05); n = 6.*

reduce the potential for worker discomfort and to directly apply the spray to conveyor B.

The application of water spray on the conveyor resulted in APC and ECC reductions of 0.51 ± 0.75 and 0.34 ± 0.71 log CFU/100 cm^2, respectively; APC and ECC levels were not significantly different ($P > 0.05$) from those initially measured on the conveyor (5.85 ± 0.47 and 4.85 ± 0.23 log CFU/100 cm^2, respectively; *Fig. 4*). Although the conveyor was not directly sprayed with water or PAA, it was wet at the time of initial sampling because of contact with hulled walnuts. APC reductions of 1.34 ± 1.00 log CFU/100 cm^2 were achieved on conveyor B with an application of 100 ppm PAA spray; results were not significantly different ($P > 0.05$) from those observed with application of 25 ppm PAA (reductions of 1.53 ± 0.61 log CFU/100 cm^2). The application of all four PAA concentrations resulted in ECC levels that were not significantly different ($P > 0.05$) from each other and were all significantly lower than those measured on the conveyor sprayed with water (average reduction of 2.07 log CFU/100 cm^2). No employee complaints were received in the second trial during application of the PAA sprays, in large part because of the relocation of the spray bar.

Organoleptic analysis of walnut kernels extracted from inshell walnuts exposed to a tank of water containing 25 ppm PAA was conducted by a trained sensory panel contracted as part of a separate study *(6)*. The percent quality change in appearance, aroma, texture, flavor, and astringency was dependent on shell integrity (broken versus intact) and exposure to PAA. The walnuts in the present study were not submerged in water containing PAA. Although it is likely that the negative sensory impact on the walnuts would be lessened by PAA application to the conveyor belts, further work would be needed to confirm this assumption. Spray bars could be installed in locations that would reduce the likelihood of PAA coming into direct contact with the walnuts as they are conveyed, further reducing any impact of PAA on the sensory quality of the walnuts.

Recommended walnut huller-dehydrator practices have been published *(13)*. However, data to support more specific qualitative and quantitative operational guidance has been lacking. In this study, reductions in APC (1.34 to 1.56 log CFU/100 cm^2) and ECC (1.94 to 2.30 log CFU/100 cm^2) were achieved with applications of 25 to 100 ppm PAA onto conveyer belts in a commercial walnut huller-dehydrator under otherwise typical conditions of operation. Though not specifically evaluated in this study, the application of PAA sprays may reduce the potential for conveyer belt surfaces to serve as transfer points for cross-contamination within and between loads of walnuts. Walnut handlers must consider the potential impact of the application of PAA to conveyor belts on walnut kernel quality and employee comfort, as well as the added costs of the equipment, water, and sanitizers, when deciding whether to install a PAA spray system to supplement existing individualized food safety programs.

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