Efficacy of Aqueous and Alcohol-Based Quaternary Ammonium Sanitizers for Reducing *Salmonella* in Dusts Generated in Almond Hulling and Shelling Facilities

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ABSTRACT: Large volumes of fine particulate matter or "dust" (soil, hulls, and shells) generated when hulls and shells are removed from almond kernels complicate cleaning and sanitation procedures in the huller-sheller (HS) environment. This study evaluated the efficacy of 3 aqueous quaternary ammonium sanitizers (AQuats) and an isopropyl alcohol-based quaternary ammonium sanitizer (IPAQuat) for reducing *Salmonella* in dust collected from 2 HS facilities. Dust (1 g) was thoroughly mixed with 1 to 2 mL of inoculum (1 to 5 log CFU/g) before adding 1 to 7 mL of water, an AQuat (200 or 1000 ppm), or IPAQuat (200 ppm, 58.6% isopropyl alcohol) and incubated at 15 and 30 °C for up to 21 d. At either 15 or 30 °C increases in *Salmonella* populations in the dust were not significantly different following addition of either water or AQuats. No significant differences were observed upon water or AQuat addition, either among the 3 AQuats tested, the concentration or volume of AQuat, or the initial level of *Salmonella*. When IPAQuat was added to dust inoculated at 1 to 7 log CFU/g, *Salmonella* levels were reduced to less than 1.3 log CFU/g after treatment and after incubation at 30 °C for 48 h. IPAQuat was an effective sanitizer compared to the AQuats, even in the presence of high levels of organic material. Recent large-scale outbreaks of salmonellosis with low-moisture foods have increased concerns regarding their safety. Little research or guidance is available on appropriate cleaning and sanitation programs for these food types. This research is focused on an evaluation of sanitation options for low-moisture foods, in particular almonds. The information should be applicable and useful to the nut industry and to other low-moisture foods.

Practical Application: Recent large-scale outbreaks of salmonellosis with low-moisture foods have increased concerns regarding their safety. Little research or guidance is available on appropriate cleaning and sanitation programs for these food types. This research is focused on an evaluation of sanitation options for low-moisture foods, in particular almonds. The information should be applicable and useful to the nut industry and to other low-moisture foods.

Keywords: almonds, dust, quaternary ammonia, *Salmonella*, sanitizers

Introduction

Over the past decade, salmonellosis has increasingly been associated with low-moisture foods (Scott and others 2009) including raw almond kernels (CDC 2004; Isaacs and others 2005), peanut butter (CDC 2007, 2009), and cereals (CDC 1998, 2008). Dust has long been recognized as a means to spread *Salmonella* in animal production and in animal feeds (Miura and others 1964; Letellier and others 1999; Mitchell and others 2002; Jones and Richardson 2004) and as a vector for the spread of *Salmonella* and *E. coli* O157:H7 in humans (Bate and James 1958; Varma and others 2003).

In California, almonds are harvested mechanically by shaking them from the tree. After drying on the ground for 7 to 10 d, the almonds are swept into windrows, which are mechanically collected from the orchard floor while pressurized air is used to remove light debris. The hull and shell of the almond is separated from the kernel in huller-sheller (HS) facilities. The hulls or both the hulls and shells are removed through a series of sheer rollers prior to sending the in-shell almonds or kernels to an almond processor.

During hulling and shelling of almonds, fine particulate matter is generated that is comprised mainly of soil, hull, and shell. This "dust" is generated in significant amounts within minutes of equipment start up and, in general, is extremely difficult to eliminate from the HS environment given existing equipment and facility design (Du and others 2007). Dust may be controlled by ducting to a cyclone or fabric filter for collection and disposal, or removed by periodic application of pressurized air to blow dust from walls and equipment followed by collection and disposal (Almond Hullers and Processors Assoc. 2004). The current equipment is not designed to be broken down for cleaning except at the end of the season, which runs from July through November or December, thus providing opportunity for transfer of dust within and between lots.

Data validating the efficacy of cleaning and sanitation protocols in low-moisture food facilities is generally difficult to find and is limited to cereals, grains, and their products (Troller 1993; Umland and others 2003). A previous study in our laboratory evaluated cleaning and sanitation treatments under laboratory conditions

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using materials from HS facilities (Du and others 2007). Although water cleaners and aqueous sanitizers were shown to be very effective at reducing microbial populations under laboratory conditions, there was concern regarding their use except in the off- or preseason when equipment can be disassembled, dust completely removed and drying time is adequate.

The high soluble sugar content of almond hulls make them an excellent nutrient source for animals (Aguilar and others 1984), and the majority of the hulls produced in California are sold for dairy cattle feed. While *Salmonella* does not multiply in dry dust or on dry almond components, previous research has demonstrated that *Salmonella* can grow rapidly in wet hulls (Uesugi and Harris 2006; Danyluk and others 2008a). The growth of *Salmonella* could also be observed in soils after application of hull extract (Danyluk and others 2008b).

Given the persistence of dust in the almond hulling and shelling environment, the objectives of this study were to (1) determine the effect of water on the survival of bacteria and *Salmonella* in HS almond dust, and (2) evaluate the efficacy of 3 aqueous quaternary ammonium sanitizers (AQuat) and an isopropyl alcohol-based quaternary ammonium sanitizer (IPAQuat) for reducing *Salmonella* in almond dust.

Materials and Methods

Almond dust

Almond dust samples (hull, shell, and soil particulate materials) were collected from 5 locations in a HS facility (D-1 through D-5) and 1 location (D-6) in a 2nd HS facility in California. Dust samples from each location were stored at ambient temperature (24 ± 2 °C) in separate polyethylene bags (30.5 × 30.5 cm, Bitran; ComPac Intl., Carbondale, Ill., U.S.A.), which were sealed and placed inside a sealed plastic tub.

Characterization of almond dusts

Triplicate samples of dust (1 g) from each location were evaluated for volume, water activity using a water activity meter (Decagon Devices, Inc., Pullman, Wash., U.S.A.), and also for water absorption. To measure volume, dust samples (1 g) were weighed into 15-mL conical centrifuge tubes (BD Biosciences, Bedford, Mass., U.S.A.) without tapping or shaking, and milliliters per gram of dust were recorded for each sample. To determine the amount of water required to saturate 1 g of dust, water was added to each tube in 1-mL increments and mixed thoroughly after each addition using a vortex mixer. Water was added up to the point where the dust sample was completely wetted, but before a layer of water appeared on top of the wet dust mixture.

Microbiological analysis of dust samples

The 6 different dust samples were evaluated separately to determine the microbial populations. Triplicate samples from each location were analyzed for total aerobic plate count and *E. coli*/coliform counts. Dust samples (1 g) were added to 19 mL of Butterfield's phosphate buffer (BPB) and vortexed. After serial 10-fold dilutions in BPB, samples were plated in duplicate onto aerobic plate count (APC) 3M Petrifilm and *E. coli*/coliform count (ECC) plates (3 M Microbiology Products, St. Paul, Minn., U.S.A.). APC and ECC plates were incubated for 48 h at 30 and 37 °C, respectively.

Determination of sugar concentrations in almond dusts

Sterile distilled water (19 mL) was added to triplicate 1-g samples of almond dust in 50-mL conical centrifuge tubes to make slurries. The tubes were then placed on a refrigerated Lab-Line shaker (Barnstead Int., Dubuque, Iowa, U.S.A.) and rotated at 150 rpm for 24 ± 2 h at 4 ± 2 °C. Following agitation, each slurry was filtered through 8 layers of sterile cheesecloth to obtain a supernatant, which was stored for up to 24 h at 4 ± 2 °C until testing. Sucrose, D-glucose, and D-fructose concentrations in the supernatant were determined using an Enzymatic BioAnalysis kit (r-biopharm GmbH, Darmstadt, Germany) according to the manufacturer's instructions; D-glucose was determined before and after the enzymatic hydrolysis of sucrose, and then D-fructose was determined.

Inoculum preparation

The *Salmonella* Enteritidis PT 30 (ATCC BAA1045) and *Salmonella* Enteritidis PT 9c strains used for this study were associated with raw almond outbreaks in 2000 to 2001 and 2003 to 2004, respectively (CDC 2004; Isaacs and others 2005). *Salmonella* Typhimurium (LJH738) and *Salmonella* Montevideo (LJH654) were isolated from raw almonds in a statewide survey (Danyluk and others 2007). *Salmonella* Newport (MDD302) was an environmental isolate identical to that associated with a 2005 fresh tomato outbreak (Greene and others 2008).

Using methods described by Parnell and others (2005), variants of the *Salmonella* strains resistant to 50 μg/mL of nalidixic acid were isolated and used for this study. Growth curves of the parent and variant strains were similar in tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks, Md., U.S.A.) (data not shown). Nalidixic acid resistant strains were stored at −80 °C in TSB supplemented with 50 μg/mL nalidixic acid (Sigma, St. Louis, Mo., U.S.A.) and 15% glycerol (wt/vol).

Prior to each experiment, frozen stock culture was streaked onto tryptic soy agar (Difco, Becton Dickinson) supplemented with 50 μg/mL nalidixic acid (TSAN) and plates were incubated at 35 ± 2 °C for 24 ± 2 h. Previous research with almonds indicated that the inoculum preparation survived better when prepared on agar than in broth (Uesugi and others 2006). Therefore, a single isolated colony was selected and suspended in 10 mL of sterile deionized water to generate an inoculum of 10^7 CFU/mL. This bacterial stock solution was further diluted in sterile deionized water to generate inocula of 1 to 7 log CFU/mL.

Effect of water volume on microbial populations in dust

To determine the potential growth of aerobic bacteria and coliforms in wetted dust, 1-g dust samples (D-4 and D-6) were weighed into 50-mL conical centrifuge tubes, and sterile deionized water was added in predetermined amounts (0 to 7 mL for D-4; 0 to 3 mL for D-6) and mixed thoroughly with a vortex mixer. All dust samples (dry and wet) were kept at 30 °C for 48 h. This temperature was chosen to simulate typical conditions in HS facilities during seasonal operations. A corresponding amount of BPB was then added to each dust sample to bring the total volume of liquid added per tube to 19 mL. After serial 10-fold dilution, 1-mL aliquots were plated in duplicate onto APC and ECC plates and incubated for 48 h at 30 and 37 °C, respectively.

A similar protocol was used to determine the potential growth of *Salmonella* in wetted dust. Two sets of 1-g dust samples (for both D-4 and D-6) were weighed into 50-mL conical centrifuge tubes, and *Salmonella* inoculum (approximately 4 log CFU/mL) was added (1 to 7 mL for D-4; 1 to 3 mL for D-6) to triplicate samples of each dust. After mixing thoroughly with a vortex mixer, 1 set of samples was analyzed immediately and the other set was held at 30 °C for 48 h before analysis. Following incubation, a corresponding amount of BPB was added to each dust sample to bring the total volume

of liquid added per tube to 19 mL. After serial 10-fold dilution, 0.1 mL-aliquots were plated in duplicate onto TSAN plates and bismuth sulfite agar (BSA; Difco, Becton Dickinson) supplemented with 50 μg/mL nalidixic acid (BSAN) plates. If the count was predicted to be 1 log CFU/g or less, then a 1-mL aliquot was plated over 5 plates (0.2 mL each) of TSAN and BSAN. TSAN and BSAN plates were incubated at 37 °C for 24 and 48 h, respectively.

Sanitizers

Three aqueous quaternary ammonium (AQuat) sanitizers (A, B, and C) and an isopropyl alcohol-based quaternary ammonium sanitizer (IPAQuat) were evaluated because they were in use at several HS facilities in California at the time this study was initiated. Each sanitizer was prepared and used according to the manufacturer's instructions. AQuat-A (200 or 1000 ppm) was prepared by adding 0.5 or 2.5 mL of 3M Sanitizer concentrate (3M, St. Paul, Minn., U.S.A.) to 50 mL of sterile deionized water. For AQuat-B (200 ppm), 0.13 mL of Quat Guard (Unisource Worldwide, Atlanta, Ga., U.S.A.) was added to 50 mL of sterile deionized water; for AQuat-C (200 ppm), 0.10 mL of Sani-T-10 (Spartan Chemical Co., Maumee, Ohio, U.S.A.) was added to 50 mL of sterile deionized water. Final concentrations of all AQuat solutions were confirmed using a QAC strip (Weber Scientific, Hamilton, N.J., U.S.A.). The IPAQuat (Alpet D2; Best Sanitizers Inc., Penn Valley, Calif., U.S.A.) was obtained premixed (200 ppm, 58.6% isopropyl alcohol).

Effect of AQuat sanitizers on survival of *Salmonella*

The effect of AQuat sanitizers on *Salmonella* was first determined in a water suspension by mixing 1 mL AQuat-A (200 ppm) or sterile deionized water (control) with 2 mL of *Salmonella* inoculum (5 or 7 log CFU/mL). Samples were allowed to stand for 1 min before analysis. To compare the antimicrobial effects of the different aqueous sanitizers in dust, D-6 samples (1 g) were mixed with *Salmonella* inoculum (1 or 2 mL) at concentrations from 1 to 5 log CFU/mL, and these samples were then treated with specific volumes of Aquat-A (at 200 or 1000 ppm), B or C (200 ppm). Treated samples were analyzed immediately and after storage at 30 °C for 48 h, or up to a week, using the enumeration procedures described previously. In a separate temperature study, dust samples treated with AQuat-A (1 mL, 200 ppm) were stored at 15 or 30 °C for up to 21 d, and 3 samples from each set were removed from storage at different times for microbial analysis.

Effect of aqueous and alcohol-based sanitizers on bacteria in dust

Dust D-4 and D-6 samples (1 g) were mixed thoroughly with either 2 mL of sterile deionized water or 2 mL of *Salmonella* inoculum (4.5 log CFU/mL) before adding 1 mL of treatment solution: sterile water, AQuat-A (200 ppm), or IPAQuat (58.6% IPA). These dust mixtures were vortexed vigorously and held at room temperature for 10 to 15 min. One set of samples was analyzed immediately and the other set was stored at 30 °C for 48 h before analysis, using the same enumeration procedures described previously. Triplicate samples were used for each experiment; each experiment was repeated once.

Effect of isopropyl alcohol concentration on *Salmonella* in dust

To further assess the antimicrobial effect of isopropyl alcohol, dust D-6 samples (1 g) were mixed thoroughly with 2 mL of *Salmonella* inoculum (5 log CFU/mL) before adding 1 mL of 10%, 20%, 30%, 40%, 50%, or 60% isopropyl alcohol (IPA, diluted in sterile water, v/v) or sterile water (control). The dust mixtures were vortexed vigorously and held at room temperature for 10 to 15 min. Treated samples were either analyzed immediately or after storage at 30 °C for 48 h.

Statistical analysis

Data were analyzed using Statistical Analysis System (version 8.2) software (SAS Inst., Cary, N.C., U.S.A.). Analysis of variance by the General Linear Model procedure and Duncan's multiple range tests were used to find the significant differences ($P < 0.05$) among treatments.

Results and Discussion

Almond dust characteristics

A visual examination of the dust samples collected from different locations in HS facilities revealed differences in the proportion and sizes of the components, which were assumed to be hull, shell, and soil particles. The dry dust samples varied in volume from 2.5 to 7.9 mL/g, and the water activity of the samples ranged from 0.41 to 0.46 (Table 1). The amount of water required to saturate 1-g dust samples ranged from 4 mL for D-1, D-4, and D-5 (highest volume dust) to 2 mL for D-6 (lowest volume dust). All dust samples had similar aerobic plate counts (APC) and coliform counts that ranged from 5.8 to 6.8 log CFU/g and 3.8 to 5.2 CFU/g, respectively (Table 1). Levels of presumptive *E. coli* were <1.3 log CFU/g (D-2 and D-6) to 2.5 to 2.8 log CFU/g (D1 and D3-D5). Counts of uninoculated dust samples were below the limit of detection (<1.3 log CFU/g) on BSAN; colonies of naturally-occurring bacteria (unknown identity) were occasionally detected on TSAN. In most cases the counts for inoculated samples on TSAN and BSAN were not significantly different (data not shown); however, to avoid interference with the background population, all *Salmonella* data shown for inoculated samples are for counts obtained on BSAN.

Table 1 — Analysis of dust samples collected from different locations in huller-sheller facilities ($n = 3$).

Dust	Volume (mL/g)	Water activity[a]	Water to saturate (mL)	Microbial populations (log CFU/g)			Sugar concentrations (g/g)[c]		
				APC	Coliform	*E. coli*[b]	Sucrose	D-glucose	D-fructose
1	5.3 ± 0.2	0.46	4	5.9 ± 0.2	3.9 ± 0.2	2.7 ± 0.1	0.26 ± 0.02	0.08 ± 0.01	0.28 ± 0.01
2	4.0 ± 0.3	0.43	3	6.2 ± 0.2	3.8 ± 0.2	<1.3[d]	0.15 ± 0.02	0.01 ± 0.02	0.17 ± 0.02
3	3.7 ± 0.3	0.45	3	5.8 ± 0.2	4.3 ± 0.2	2.5 ± 0.0	0.23 ± 0.00	0.00 ± 0.02	0.15 ± 0.00
4	7.9 ± 0.5	0.44	4	6.3 ± 0.5	5.2 ± 0.5	2.7 ± 0.6	0.17 ± 0.00	0.03 ± 0.00	0.18 ± 0.01
5	6.0 ± 0.1	0.43	4	6.2 ± 0.2	4.5 ± 0.2	2.8 ± 0.0	0.10 ± 0.01	0.02 ± 0.01	0.16 ± 0.01
6	2.5 ± 0.2	0.41	2	6.8 ± 0.1	5.0 ± 0.3	<1.3	0.08 ± 0.01	0.07 ± 0.00	0.14 ± 0.00

[a]Standard deviation < 0.01.
[b]Presumptive *E. coli*; not confirmed.
[c]Reported as grams of soluble sugar per gram of dry dust based on enzymatic determination.
[d]Detection limit = 1.3 log CFU/g.

Sugar concentrations in almond dusts

The hull and shell components of the almond dust provided soluble sugars for bacterial growth; the concentration of these sugars varied substantially with each dust sample (Table 1). Sucrose, D-glucose, and D-fructose levels of 8% to 26%, 0% to 8%, and 14% to 28% of the total dry weight of the dust, respectively, were observed. Total levels of the soluble sugars in the almond dusts collected in California and used in this study ranged from 28% to 63%, higher than levels described previously for hulls (Saura-Calixto and Cañellas 1982). Almond shells provide limited soluble sugar (0.35%) on a dry weight basis (Saura-Calixto and others 1983) compared to hulls, which have higher levels of soluble sugar (26.6%) made up of sucrose (40%), glucose (23%), and fructose (17%) (Saura-Calixto and Cañellas 1982). The values determined by Saura-Calixto and Cañellas (1982) were from a mixture of principal almond varieties in Spain, which may account for the observed differences in sugar levels. No record was made of almond varieties that were being hulled and shelled prior to the collection of dust samples used in this study. Based on the level of soluble sugars detected in almond dust samples, and the limited concentration of soluble sugars present in almond shells, it is likely that the almond dust samples used in this study were comprised primarily of almond hull material.

Growth of bacteria in dusts wetted with water

Growth of aerobic bacteria, coliforms, and *Salmonella* Enteritidis PT 30 was observed after 48 h at 30 °C in almond dusts wetted with sufficient water (Table 2). APC increased significantly ($P < 0.05$) by 3 log CFU/g in dusts wetted with ≥2 mL water per gram. Coliform levels also increased significantly ($P < 0.05$) by 2 to 4 log CFU/g in dust wetted with ≥3 or ≥2 mL water per gram for D-4 or D-6, respectively. *Salmonella* Enteritidis PT 30 increased significantly ($P < 0.05$) in inoculated dust wetted with ≥3 mL water per gram for D-4 or ≥2 mL water/g for D-6. These levels of added water were at or below the saturation point (Table 1). For subsequent experiments, dust samples were wetted with 1 to 2 mL of liquid (inoculum or water) before adding 1 to 2 mL of the treatment solution.

Effect of temperature and AQuat type, volume, and concentration on survival of *Salmonella* in dust wetted with AQuat-A

Growth of *Salmonella* Enteritidis PT 30 was similar in dust D-6 treated with sterile deionized water or AQuat-A. A maximum density was achieved after approximately 48 h of incubation (Figure 1) at 30 °C. When inoculated and wetted dust was incubated at 15 °C, growth was not evident for 76 h (Figure 1). Thereafter, in both the AQuat and water-treated samples, the population increased approximately 2 log CFU/g by day 6 and remained constant over the next 15 d. For all subsequent experiments samples were incubated for 48 h at 30 °C.

The presence of AQuat-A reduced *Salmonella* Enteritidis PT 30 populations by >7 log CFU/mL within 1 min when combined in a deionized water suspension (data not shown). However, in dust D-6, treatment with 2 mL of either water or aqueous-based

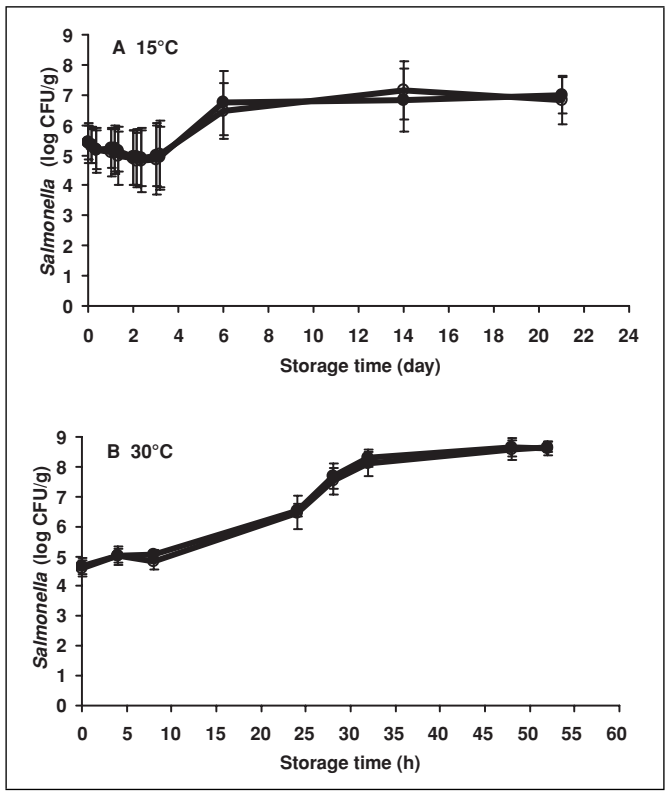

Figure 1—Survival of *Salmonella* Enteritidis PT 30 (1 mL inoculum) in dust D-6 wetted with 1 mL sterile water (•) or 200 ppm AQuat-A (○) and stored at 15 or 30 °C; plated on BSAN ($n = 6$).

Table 2—Survival of aerobic bacteria (APC), coliforms, and *Salmonella* Enteritidis PT 30 Nal^r (log CFU/g) in almond dust wetted with different volumes of water or inoculum and stored at 30 °C.

Water or inoculum added (mL)[a]	APC 48 h	Coliforms 48 h	*Salmonella* 0 h	*Salmonella* 48 h
Dust D-4				
0	5.7 ± 0.2 A[b]	2.5 ± 0.5 A	ND[c]	ND
1	5.8 ± 0.1 A	3.9 ± 1.0 AB	3.8 ± 0.1 a[d]	3.5 ± 1.2 a
2	7.4 ± 0.4 B	4.2 ± 1.6 AB	4.2 ± 0.2 a	5.0 ± 0.6 a
3	8.9 ± 0.2 D	5.4 ± 0.5 BC	4.2 ± 0.0 a	5.9 ± 0.6 b
4	8.9 ± 0.1 D	6.7 ± 1.2 C	4.3 ± 0.1 a	6.8 ± 0.7 b
5	8.4 ± 0.1 C	6.6 ± 0.1 C	3.9 ± 0.2 a	5.8 ± 0.5 b
6	8.6 ± 0.1 C	7.0 ± 0.1 C	4.1 ± 0.1 a	5.5 ± 0.9 b
7	8.7 ± 0.1 C	6.7 ± 0.1 C	4.2 ± 0.0 a	5.1 ± 0.9 b
Dust D-6				
0	6.8 ± 0.1 B	4.8 ± 0.2 A	–	–
1	6.4 ± 0.1 A	4.2 ± 0.4 A	3.1 ± 0.1 a	1.7 ± 0.8 b
2	9.1 ± 0.1 C	6.6 ± 0.3 B	3.3 ± 0.0 a	6.0 ± 0.0 b
3	9.3 ± 0.2 C	7.3 ± 0.5 C	3.7 ± 0.3 a	6.3 ± 0.1 b

[a]Volume of sterile water (for APC and coliforms) or *Salmonella* inoculum added to 1-g dust samples.
[b]Within columns and dust type, mean values (± SD) with different uppercase letters are significantly different ($P < 0.05$); $n = 3$.
[c]ND, not determined.
[d]Within rows, mean values (± SD) with different lowercase letters are significantly different ($P < 0.05$).

sanitizer (A, B, or C) at 200 ppm resulted in an increase in *Salmonella* populations by 4.3 to 4.7 log CFU/g after 48 h of incubation at 30 °C (data not shown). Since the results from the AQuats were not significantly different ($P > 0.05$), further studies on the efficacy of aqueous-based sanitizers used only AQuat-A.

Increasing the volume of AQuat-A from 2 to 4 mL/g of dust and the concentration from 200 to 1000 ppm did not significantly improve ($P > 0.05$) the antimicrobial effect of this aqueous-based sanitizer in inoculated almond dust. After 48 h of incubation at 30 °C, the population of *Salmonella* Enteritidis PT 30 significantly increased ($P < 0.05$) by 3.3 to 4 log CFU/g of dust, regardless of sanitizer volume or concentration.

Organic matter is widely known to impact sanitizer efficacy (Troller 1993). Although quaternary ammonium sanitizers are considered less sensitive to organic materials than chlorine, these data demonstrate that dusts generated in almond HS facilities eliminate the antimicrobial properties of AQuats. Therefore, these sanitizers should only be used in HS facilities when dusts can be completely removed by thorough cleaning, typically when the facility is nonoperational and equipment can be torn down, washed, sanitized, and dried.

Influence of *Salmonella* inoculum concentration in dust treated with AQuat-A

When almond dust D-6 was inoculated with *Salmonella* Enteritidis PT 30 levels from 1 to 5 log CFU/g, the addition of water or AQuat-A (200 ppm) had no significant effect ($P > 0.05$) on growth of *Salmonella* (Table 3). The population of *Salmonella* increased by 3.5 to 4.3 log CFU/g after incubation at 30 °C for 48 h, when the initial inoculation concentration was 1.7 log CFU/g or greater, and remained at these levels during an additional week of storage. Because overall increases were similar, regardless of inoculation level, final populations in the dust ranged from 2.4 to 8.3 log CFU/g. Similar results were observed for dust D-6 inoculated with 4 additional strains of *Salmonella* (*Salmonella* Enteritidis PT 9c, *Salmonella* Typhimurium, *Salmonella* Montevideo, and *Salmonella* Newport). With initial inoculum levels of 5 log CFU/g, increases of 3.1 to 4 log CFU/g were observed 48 h after addition of water or AQuat A (200 ppm).

Survival of bacteria in dusts treated with aqueous or alcohol-based sanitizers

Aerobic bacteria and coliform counts did not differ significantly ($P > 0.05$) in dust D-4 and D-6 wetted with 2 mL of water and treated with 1 mL of water or AQuat-A; significant ($P < 0.05$) 3-log CFU/g increases were observed after 48 h of incubation at 30 °C (Table 4). With the addition of IPAQuat, a decrease of 0.6 or 1 log CFU/g was noted immediately for the population of aerobic bacteria in D-4 or D-6, respectively. After 48 h the level of aerobic bacteria did not change for D-4 and increased approximately 0.6 log CFU/g in dust D-6. Similarly, when IPAQuat was evaluated in a commercial HS facility, the APC counts were not significantly reduced on any of the almond-contact surfaces tested (Du and others 2007).

Coliform populations fell to <1.3 log CFU/g immediately after treatment with IPAQuat and remained at the limit of detection after incubation at 30 °C for 48 h (Table 4). In contrast, no significant reductions in coliform counts were observed after IPAQuat was applied in a HS under commercial conditions (Du and others 2007). During cleaning procedures observed under the commercial conditions, dust was not uniformly removed from equipment, even

Table 3 — Effect of AQuat-A (200 ppm) in dust D-6 inoculated with different concentrations of *Salmonella* Enteritidis PT 30 Nal^r and stored at 30 °C.

Concentration of inoculum added (log CFU/mL)	Treatment solution[a]	*Salmonella* (log CFU/g)[b]		
		0 h	48 h	1 wk
1	Water	1.3 ± 0.0 (4/12)[c] a[d]	3.6 ± 2.0 (6/12) b	4.1 ± 2.8 (5/9) b
	AQuat-A	1.3 ± 0.0 (1/12) a	2.4 ± 1.7 (4/12) ab	3.2 ± 2.0 (5/9) b
2	Water	1.7 ± 0.3 (6/6) a	5.3 ± 1.6 (6/6) b	5.2 ± 2.3 (5/6) b
	AQuat-A	1.7 ± 0.4 (4/6) a	5.7 ± 0.8 (6/6) b	5.6 ± 2.4 (5/6) b
3	Water	2.7 ± 0.3 a	6.6 ± 0.7 b	7.2 ± 0.4 b
	AQuat-A	2.6 ± 0.1 a	6.9 ± 0.5 b	6.8 ± 0.4 b
4	Water	3.7 ± 0.3 a	7.2 ± 0.8 b	7.7 ± 0.5 b
	AQuat-A	3.7 ± 0.3 a	7.5 ± 0.6 b	7.9 ± 0.3 b
5	Water	4.6 ± 0.2 a	8.3 ± 0.5 b	7.8 ± 0.5 b
	AQuat-A	4.6 ± 0.2 a	8.2 ± 0.5 b	7.8 ± 0.6 b

[a]Before adding treatment solution (1 mL), dust samples (1 g) were mixed with 1 mL *Salmonella* inoculum.
[b]$n \geq 6$; detection limit = 1.3 log CFU/g.
[c]Number of samples with a single *Salmonella* colony at the lowest dilution per total samples analyzed.
[d]Within rows, means (± SD) with different letters are significantly different ($P < 0.05$).

Table 4 — Survival of aerobic bacteria, coliforms, and *Salmonella* Enteritidis PT 30 Nal^r (log CFU/g, $n = 6$) in almond dusts treated with water or sanitizer and stored at 30 °C.

Treatment solution[a]	APC		Coliforms		*Salmonella*	
	0 h	48 h	0 h	48 h	0 h	48 h
Dust D-4						
Water	6.2 ± 0.4 Aa[b]	9.3 ± 0.2 Ab	4.4 ± 0.4 Aa	7.5 ± 0.6 Ab	4.6 ± 0.3 Aa	6.1 ± 0.5 Ab
AQuat-A[c]	6.2 ± 0.5 Aa	9.3 ± 0.2 Ab	4.6 ± 0.3 Aa	7.3 ± 0.6 Ab	4.6 ± 0.4 Aa	6.0 ± 0.5 Ab
IPAQuat	5.6 ± 0.2 Ba	5.7 ± 0.1 Ba	<1.3 ± 0.0 Ba	1.4 ± 0.4 Bb	2.7 ± 1.1 Ba	<1.3 ± 0.0 Bb
Dust D-6						
Water	6.8 ± 0.1 Aa	9.5 ± 0.2 Ab	5.0 ± 0.1 Aa	7.5 ± 0.3 Ab	5.2 ± 0.2 Aa	7.3 ± 0.5 Ab
AQuat-A	6.8 ± 0.1 Aa	9.6 ± 0.1 Ab	5.1 ± 0.4 Aa	7.9 ± 0.5 Ab	5.2 ± 0.0 Aa	7.5 ± 0.4 Ab
IPAQuat	5.8 ± 0.6 Ba	6.4 ± 0.2 Bb	<1.3 ± 0.0 Ba	1.3 ± 0.1 Ba	<1.3 ± 0.0 Ba	<1.3 ± 0.0 Ba

[a]Before adding treatment solution (1 mL), dust samples (1 g) were wetted with 2 mL of sterile water (for APC and coliforms) or 2 mL of *Salmonella* Enteritidis PT 30 inoculum.
[b]Within columns and dust type, means (± SD) with different uppercase letters are significantly different ($P < 0.05$); within rows and dust type, means (± SD) with different lowercase letters are significantly different ($P < 0.05$).
[c]AQuat-A concentration was 200 ppm.

application of IPAQUAT was difficult, and time between application and sampling was short, which may, in part, explain the observed lack of coliform reduction in a commercial setting.

Growth of *Salmonella* Enteritidis PT 30 was not significantly different ($P > 0.05$) in dusts D-4 and D-6 treated with 1 mL of sterile deionized water or AQuat-A; 2 log CFU/g increases were observed after 48 h of incubation at 30 °C (Table 4). However, when 1 mL of IPAQuat sanitizer was added to inoculated D-4 or D-6, a reduction of *Salmonella* to 2.7 or <1.3 log CFU/g, respectively, was observed immediately after treatment; in both samples the *Salmonella* counts were <1.3 log CFU/g after incubation at 30 °C for 48 h. Populations of *Salmonella* Enteritidis PT 9c, *Salmonella* Typhimurium, *Salmonella* Montevideo, and *Salmonella* Newport also declined 2.4 to 3.3 log CFU/g immediately after treatment with 1 mL of IPAQuat. Levels were below the limit of detection (<1.3 log CFU/g) after 48 h (data not shown). When the volume of added IPAQuat was increased to 2 mL, the *Salmonella* population fell to <1.3 log CFU/g immediately after treatment and remained at this level after incubation at 30 °C for 48 h.

Effect of isopropyl alcohol (IPA) concentration on the survival of *Salmonella*

IPAQuat is sold premixed at a concentration of 58.6% IPA and 200 ppm Quat. Adding IPA alone to dust D-6 at concentrations ≥50% resulted in an immediate reduction in *Salmonella* counts to <1.3 log CFU/g (Table 5). At IPA concentrations ≥10%, *Salmonella* populations in inoculated dust decreased by 0.5 log CFU/g after incubation at 30 °C for 48 h (Table 5). At ≥20%, *Salmonella* populations decreased to <1.3 log CFU/g after 48 h.

The outbreak strain *Salmonella* Enteritidis PT 30 was isolated from a processor and HS facility months after the implicated almonds were processed (Isaacs and others 2005). Furthermore, *Salmonella* Enteritidis PT 30 could be isolated from one of the implicated almond orchards over a 5-y period (Uesugi and others 2007). This ability of *Salmonella* to persist in dry processing facilities has been also noted for cereal (CDC 1998, 2008; Scott and others 2009), dry dog food (Scott and others 2009), and peanut butter (CDC 2007) where outbreak strains were isolated from the manufacturing facility years apart or months after initial cases were observed.

Given the potential for *Salmonella* to persist in dry processing facilities, inadvertent introduction of water (roof leaks, faulty sprinklers) is of particular concern (Scott and others 2009). Deliberate introduction of water through the use of aqueous cleaners and sanitizers should also be carefully controlled or avoided particularly when high levels of dust are anticipated (Umland and others 2003).

Table 5 — Survival of *Salmonella* Enteritidis PT 30 in inoculated dust D-6 treated with isopropyl alcohol at various concentrations and stored at 30 °C.

Treatment solution[a]	Nr of replicates	*Salmonella* (log CFU/g)[b]	
		0 h	48 h
Water	6	4.7 ± 0.2 A[c]a[d]	8.1 ± 0.8 Ab
Isopropyl alcohol			
10%	3	4.8 ± 0.0 Aa	4.3 ± 0.3 Bb
20	3	4.6 ± 0.0 Aa	<1.3 ± 0.0 Cb
30	3	3.4 ± 0.0 Ba	<1.3 ± 0.0 Cb
40	3	1.5 ± 0.3 Ca	<1.3 ± 0.0 Ca
50	3	<1.3 ± 0.0 Ca	<1.3 ± 0.0 Ca
60	3	<1.3 ± 0.0 Ca	<1.3 ± 0.0 Ca

[a]Before adding treatment solution (1 mL), dust samples (1 g) were mixed with 2 mL of *Salmonella* inoculum (5 log CFU/mL).
[b]Detection limit = 1.3 log CFU/g.
[c]Within columns, means (± SD) with different uppercase letters are significantly different ($P < 0.05$).
[d]Within rows, means (± SD) with different lowercase letters are significantly different ($P < 0.05$).

Conclusions

In HS dust, AQuats were ineffective in reducing *Salmonella* or in preventing growth of this organism. Increasing the amount or concentration of AQuats did not improve the antimicrobial efficacy over a broad range of inoculum levels. The introduction of water or use of AQuats should be restricted in HS facilities where dust cannot be strictly controlled. To avoid the multiplication of *Salmonella* in HS facilities, AQuats are only appropriate when equipment is accessible, complete removal of dust is possible with cleaning, and adequate time is available for thorough drying of the equipment.

IPAQuat reduced *Salmonella* densities by as much as 7 log CFU/g in HS dust. *Salmonella* were not detected in IPAQuat-wetted dust after 48 h of incubation at 30 °C. IPAQuat or other alcohol-based sanitizers may be appropriate for in-season HS facility sanitation programs.

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