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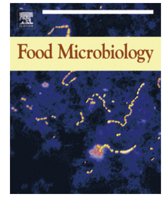

Cleaning and sanitation of *Salmonella*-contaminated peanut butter processing equipment

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ABSTRACT

Microbial contamination of peanut butter by *Salmonella* poses a significant health risk as *Salmonella* may remain viable throughout the product shelf life. Effective cleaning and sanitation of processing lines are essential for preventing cross-contamination. The objective of this study was to evaluate the efficacy of a cleaning and sanitation procedure involving hot oil and 60% isopropanol, ± quaternary ammonium compounds, to decontaminate pilot-scale processing equipment harboring *Salmonella*. Peanut butter inoculated with a cocktail of four *Salmonella* serovars (~7 log CFU/g) was used to contaminate the equipment (~75 L). The system was then emptied of peanut butter and treated with hot oil (90 °C) for 2 h followed by sanitizer for 1 h. Microbial analysis of food-contact surfaces (7 locations), peanut butter, and oil were conducted. Oil contained ~3.2 log CFU/mL on both trypticase soy agar with yeast extract (TSAYE) and xylose lysine deoxycholate (XLD), indicating hot oil alone was not sufficient to inactivate *Salmonella*. Environmental sampling found 0.25–1.12 log CFU/cm^2 remaining on processing equipment. After the isopropanol sanitation (±quaternary ammonium compounds), no *Salmonella* was detected in environmental samples on XLD (<0.16 log CFU/cm^2). These data suggest that a two-step hot oil clean and isopropanol sanitization treatment may eliminate pathogenic *Salmonella* from contaminated equipment.

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1. Introduction

Low-moisture foods, including nut butters, were once thought to be relatively safe with respect to foodborne illness risk since low water activities do not permit the growth of foodborne microorganisms. Research has shown that while pathogenic foodborne microorganisms are unable to grow in these foods, they are able to persist in the products at both refrigerated and ambient conditions for a long period of time (Burnett et al., 2000; Grasso et al., 2010; Keller et al., 2012; Park et al., 2008). Furthermore, low-moisture foods such as peanuts, almonds, hazelnuts, sesame seeds, pine nuts, walnuts, and pistachios have all been implicated in outbreaks or recalled due to contamination with bacterial pathogens (Centers for Disease Control and Prevention, 2011; U. S. Food and Drug Administration, 2004; U. S. Food and Drug Administration, 2009a; U. S. Food and Drug Administration, 2010). Nuts may become contaminated with pathogens, such as *Salmonella*, at any point from growth to processing (Schaffner et al., 2013). Since the mid 1990's there have been five recorded *Salmonella* outbreaks associated with commercial peanut butter, peanut-based products, and peanut flavored savory snacks (Centers for Disease Control and Prevention, 2007, 2009, 2012; Killalea et al., 1996; Scheil et al., 2008). More than 1150 people became ill consuming *Salmonella*-contaminated peanut butter products during two particularly high-profile U.S. outbreaks in 2007 and 2009 (Centers for Disease Control and Prevention, 2011), which highlighted the vulnerability of such products to contamination by pathogenic microorganisms. The 2012 outbreak, involving a variety of nut butter products processed at the same facility, was likely the result of poor equipment sanitation (U. S. Food and Drug Administration, 2012).

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Typical grinding and packaging of roasted peanuts occurs at 48 °C (Woodroof, 1983) yet inactivation of microorganisms in peanut butter and peanut butter products is minimal at this temperature (Mattick et al., 2001). Keller et al. (2012), found a 6 log CFU/g reduction in *Salmonella* over 50 min at 85 °C. Ma et al. (2009) and Shachar and Yaron (2006) found similar results. These findings indicate thermal processing of peanut butter is not a practical intervention step. Consequently, to reduce the risk of foodborne illness in such products, prevention of contamination rather than processing is essential.

Effective cleaning and sanitation of nut butter lines are essential for preventing both initial and cross-contamination with microbial hazards such as *Salmonella* (U. S. Food and Drug Administration, 2009b; Grocery Manufacturers Association, 2009a, 2009b, 2010; International Life Science Institute Europe, 2011; Podolak et al., 2010; Scott et al., 2009). In a May 2007 survey of Grocery Manufacturers Association (GMA) members, 53% of respondents ($n = 17$ respondents) had manufacturing periods for low-moisture areas of processing that extended 7 days or longer prior to shutting down for sanitation (Scott et al., 2009). Several companies reported production periods as long as 28–35 days prior to shutting down for sanitation (Scott et al., 2009). Environmental investigations of consecutive *Salmonella* outbreaks originating from a single bakery found that inadequately cleaned piping nozzles may have been responsible for a continual cross-contamination problem (Evans et al., 1996). Traditional wet-cleaning methods are generally not used in such a setting, as the introduction of moisture may increase the risk of equipment contamination (Beuchat et al., 2013; Codex Alimentarius, 1979). Traditional dry-cleaning methods are not suitable for all dry cleaning situations, such as nut butters (International Life Science Institute Europe, 2011). Currently there are no validated sanitation programs for use in high fat emulsified processing plants. As this type of product was considered to be of minimal risk, good manufacturing practices have not been implemented and collectively used throughout the nut butter manufacturing industry. Non-aqueous based chemical sanitation methods are needed to ensure the killing/removal of pathogenic microorganisms from nut butter processing equipment. However, the efficacy of such treatments needs further investigation. Potential non-aqueous chemical sanitation products, such as quaternary ammonium compounds or isopropyl alcohol containing quaternary ammonium compounds, have shown promising effects against *Salmonella* in low-moisture environments (Du et al., 2007, 2010; Kamineni et al., 2011). A range of products are commercially available for disinfection of low-moisture food production areas. Isopropanol has the added benefit of evaporating quickly and leaving no residue on food production surfaces. The objective of this research was to evaluate the efficacy of a model cleaning and sanitation method involving 60% isopropanol with and without the addition of quaternary ammonium compounds on *Salmonella* removal from pilot-scale peanut butter processing equipment.

2. Methods and methods

2.1. Organisms and growth conditions

Salmonella Tennessee K4643 and *Salmonella* Anatum 5802 were obtained as a gift from Dr. L. Beuchat, University of Georgia (Athens, GA). *S.* Tennessee K4643 was originally isolated from the 2006 United States peanut butter outbreak (Centers for Disease Control and Prevention, 2007). *S.* Anatum 5802 was isolated from a raw pecan sample. *Salmonella* Typhimurium 09-0001-E5 and *Salmonella* Typhimurium 09-0001-A1 cultures were obtained as a gift from the Minnesota Department of Agriculture (St. Paul, MN) and originally isolated from peanut butter involved in a 2008 multistate outbreak in the United States. Stock cultures were maintained frozen at −20 °C.

Overnight cultures of the four *Salmonella* serovars were transferred from TSAYE to test tubes containing 10 mL trypticase soy broth (TSB; Becton, Dickinson, and Co.). After 24 h incubation (37 °C), 0.1 mL was spread on the surface of TSAYE plates and incubated for 24 h at 37 °C to obtain lawn cultures. After incubation, cells were harvested from plates by adding 1 mL TSB and mixed using a sterile L-shaped plate spreader (Fisher Scientific, Pittsburgh, PA). The suspension was removed from the plate using a 1-mL pipette and collected in a 50 mL conical centrifuge tube (Becton Dickinson, and Co., Franklin Lake, NJ). Approximately 0.5 mL cell suspension was recovered from each plate. Ten plates of each serovar were harvested separately for each experiment. Approximately 20 mL of cell suspension was recovered from 40 plates. Five mL of each serovar cell suspension was pipetted together to form the *Salmonella* cocktail used in this study. The concentration of harvested cells was 11.6 ± 0.6 log CFU/mL. This cell suspension was then mixed 1:1 with peanut oil and approximately 1 mL of Tween 80 (Fisher Chemical, Whippany, NJ).

The cell:oil:Tween 80 mixture was thoroughly mixed with a vortex mixer. The mixture, ~41 mL total, was equally divided between two 400 g lots of commercial creamy peanut butter in separate Whirl-Pak bags (24 oz, Nasco, Fisher Scientific, Pittsburgh, PA) and mixed by alternating hand mixing and stomaching (Stomacher™ 400, Seward Ltd, West Sussex, UK) at 30 s intervals at 250 rpm three times each. These two contaminated lots were subsequently used to contaminate peanut butter used in pilot plant processing experiments.

2.2. Peanut butter processing equipment

A peanut butter pumping machine consisting of two steam-jacketed vessels and two pumps, with stainless steel piping (inner diameter 1.5″) to circulate molten peanut butter (donated by North Carolina State University and funded through a FDA Centers of Excellence grant awarded to Western Center for Food Safety, University of California at Davis) was installed into the IFSH Bio-Containment Pilot Plant (BCPP) to allow safe experimentation with large quantities of pathogen (Fig. 1). Biohazard suits with breathing air were used for trials involving large quantities of pathogenic microorganisms or procedures that presented a risk of aerosolization. The steam-jacketed vessels (capacity ~75 L) included thermocouple units to provide continuous in-tank temperature profiles of the heating process. The positive displacement pump from the peanut butter tank allows re-circulation of product, or disposal from system and had a flow rate of ~17.4 L/min.

2.3. Processing

Approximately 75 L of soybean oil, purchased from a wholesale supplier (Gordon Food Services, Chicago, IL), was added to the steam-jacketed tank 1 (T1) and heated to ~93 °C (Fig. 1).

Oil heated in T1 was pumped through the piping, via the centrifugal pump until the exiting oil was visibly clear (~5 L), to flush out any liquid or debris that may have been present in the piping prior to the start of each trial. Rinse oil was disposed of by pumping to a collection tank. Once clear, the remaining oil was re-circulated through the piping back to T1.

Approximately 63.5 kg of creamy peanut butter, purchased from a manufacturer in bulk containers (15.9 kg each), was autoclaved at 121 °C for 30 min (total run time 1:08 h) to soften the peanut butter for ease of handling prior to each trial. Immediately following autoclaving, the temperature of the peanut butter was ~55–60 °C, as measured by a digital thermometer probe inserted near the middle

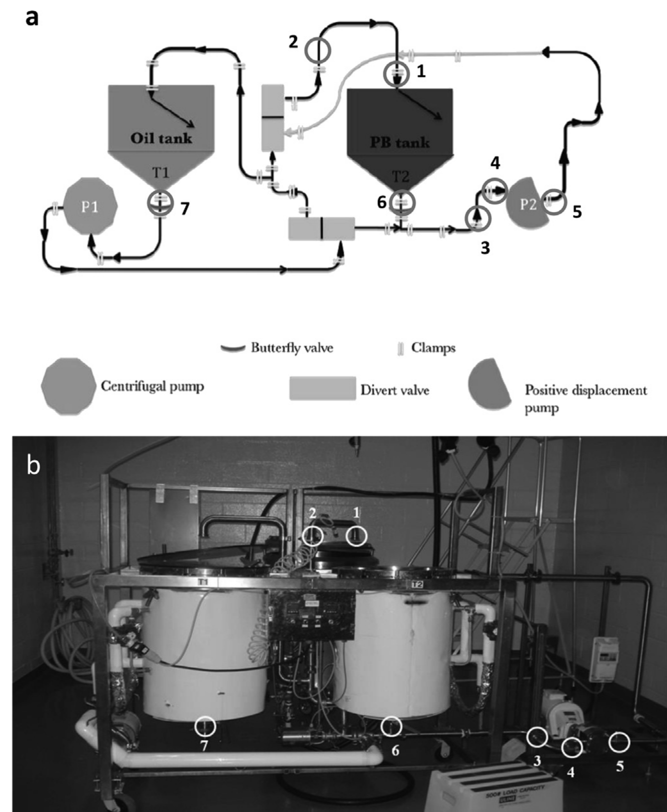

Fig. 1. a. A schematic diagram of the peanut butter pumping machine. b. Locations of environmental samples taken from the machine. 1. Exit piping; 2. Upper elbow; 3. Left elbow of lower pipe, left of positive displacement pump; 4. Right side of lower pipe, immediately before positive displacement pump; 5. Lower pipe, immediately after positive displacement pump; 6. Bottom exit of T2; 7. Bottom exit of T1.

of the container. Peanut butter treated in this manner was softened, but otherwise unchanged in character. After autoclaving, peanut butter in each container was sampled for initial bacterial counts, and then mixed with a metal 5-gal spiral mixer (Model #SM5HD, Allway Tools, Bronx, NY) attached to an electric hand drill (DW130V, Dewalt, Baltimore, MD) for approximately 1 min. Four containers of softened creamy peanut butter were emptied into the steam-jacketed tank 2 (T2), and heated to ~65 °C. Agitation was provided inside T2 by the spiral mixer. While heating in T2, the peanut butter was pumped at a flow rate of 17.4 ± 0.3 L/min from T2 through the piping to flush out any remaining oil. Peanut butter was discarded until peanut butter of a regular consistency was observed exiting the equipment (~5 L removed). Peanut butter was then re-circulated through the piping and T2 until a holding temperature of 65 °C was reached, at which time approximately 30 g of peanut butter was removed in triplicate from the exit piping (Fig. 1, #1), for determination of both microbial populations and water activity.

2.3.1. Inoculation

Approximately 60 kg heated peanut butter was inoculated with two 400-g lots of the *Salmonella*-inoculated peanut butter (described earlier) and mixed as described earlier and by re-circulating the mixture through the piping for 15 min, to ensure a fully contaminated processing line. Final *Salmonella* populations in the product were determined in triplicate samples removed at the end of the 15 min mixing period. Remaining contaminated peanut butter was then pumped into a waste container.

After the peanut butter was pumped from the system, a rinse using approximately 20 L of soybean oil at ambient temperature (~22 °C) was added directly to T2 to remove any large peanut butter clumps still remaining in the system. This initial oil rinse was re-circulated through the piping and T2 for 5 min. After 5 min the oil/peanut butter mixture was sampled in triplicate for microbial testing, and the remaining mixture was discarded.

2.3.2. Hot oil clean

Soybean oil heated to 93 °C in T1 was pumped to T2 to begin a "hot oil" cleaning procedure. The hot oil was recirculated through the piping and T2 for 5 min to ensure thorough mixing. After the initial 5 min recirculation, a '0 time' sample of the oil was taken. Subsequent samples of oil were taken at regular intervals up to 2 h (0, 30, 60, 90, and 120 min). The oil was then discarded, and environmental samples (swabs) taken as described Section 2.4.

2.3.3. Chemical sanitation

Two types of sanitation protocols, both containing isopropanol, were utilized. Equipment was cooled for 24 h prior to any chemical sanitation to ensure that isopropanol was not used near its flash point (40 °C). All experimental trials were repeated three times for each sanitizer.

2.3.3.1. Isopropanol sanitation. Isopropanol (Fisher Scientific, Pittsburgh, PA) was diluted to a 60% (v/v) concentration using deionized water. Approximately 75 L of 60% isopropanol was poured into T2. Pumping commenced, and the alcohol, which initially had a cloudy appearance due to residual oil and peanut butter in the system, was diverted to a waste container until it appeared clear (~7.5–10 L). The remaining isopropanol (~65 L) was re-circulated for 60 min at room temperature, and then diverted to waste. Environmental samples were taken as described in Section 2.4 (Fig. 1b).

2.3.3.2. Isopropanol with added quaternary ammonium compounds sanitation. Redi san RTU hard surface sanitizer (Ecolab, St. Paul, MN) containing 59% isopropanol and 0.02% quaternary ammonium compounds was used. Redi san is commercially-available and EPA-registered for food-contact surfaces, when used as the manufacturer instructs. Approximately 65 L of the commercial sanitizer was circulated through the piping and T2. Sanitizer visibly contaminated with peanut butter was diverted to a waste container until it appeared clear (~5 L). The remaining isopropanol based sanitizer was again re-circulated for 60 min at room temperature, and then diverted to waste. Environmental samples were taken as described in Section 2.4.

2.4. Environmental sampling

Environmental samples were taken at five time points during the experiment; prior to the start of the experiment, after hot oil treatment, after cooling, immediately prior to sanitizer treatment, and immediately following any sanitizer treatment. Sampling sites were as follows: 1. Exit piping of tank 2 (T2); 2. Elbow of the upper recirculation pipe of T2; 3. Left elbow of lower pipe, left of positive displacement pump; 4. Right side of lower pipe, immediately before positive displacement pump; 5. Lower pipe, immediately after positive displacement pump; 6. Bottom exit of T2; and 7. Bottom exit of tank 1 (T1) (Table 1, Fig. 1b). These sites were chosen as the most likely to contain detectable levels of *Salmonella* after the cleaning and sanitation process. All sampling sites with the exception of the bottom exit of tank 1 (T1) had direct contact with the contaminated peanut butter. Sample times and location were the same for each trial.

Environmental samples were taken by swabbing an area of ~6.25 cm^2 (2.5 cm × 2.5 cm) at the predetermined locations.

Table 1
Environmental sampling sites and rationale for sampling site inclusion.

Sample number	Environment sampling site	Rationale	Direct contact with peanut butter/sanitizer
1	Exit piping of tank 2 (T2)	This would be where product was filled into packaging	Yes
2	Elbow of the upper recirculation pipe of T2	Complete fill of this angle of pipe may be difficult and have a decreased contact-time	Yes
3	Left elbow of lower pipe, left of positive displacement pump	Higher chance or contamination due to gravitational force leading towards positive displacement pump	Yes
4	Right side of lower pipe, immediately before positive displacement pump	Higher chance or contamination due to gravitational force leading towards positive displacement pump	Yes
5	Lower pipe, immediately after positive displacement pump	Small voids may occur due to slippage of positive displacement pump leading to lower contact-time	Yes
6	Bottom exit of tank 2 (T2)	All product/sanitizer leaving the heated vessel exits through this port, higher chance or contamination and longer contact-time	Yes
7	Bottom exit of tank 1 (T1)	Contamination should not occur unless there were pump failure issues	No

The limit of detection was 0.16 log CFU/cm^2. A commercial environmental swab consisting of a five-inch, rayon-tipped swab and containing a neutralizing buffer (3M™, St. Paul, MN) was used. Environmental swabs were stored on ice (less than 2 h) until microbial analysis.

2.5. Microbial analysis

To determine *Salmonella* populations in peanut butter and oil samples, 10 g samples were taken and serially diluted 1:10 in 0.1% peptone water (Becton, Dickinson, and Co.) and plated on TSAYE and xylose deoxycholate agar (XLD, Becton, Dickinson, and Co.). One to two drops of Tween 80 was added to the first of each dilution series with the peanut butter or oil. Environmental samples were serially diluted in 0.1% peptone water and plated onto TSAYE and XLD. Plates were incubated at 37 °C for 24 h. For each trial, a few presumptive positive colonies were picked and confirmed as *Salmonella* species using Gram negative cards in the Vitek® 2 Compact (bioMérieux, Marcy l'Etoile, France).

2.6. Statistical analysis

One-way analysis of variance (ANOVA) and paired *t* tests between sampling points for the environmental swabs and also between product contamination from inoculation through hot oil treatment were calculated with SAS 9.2 (SAS Institute, Inc, Cary, NC) at a significance level of 95% ($\alpha = 0.05$).

3. Results and discussion

Although traditional water-based cleaning and sanitizing methods are very effective at reducing the presence of microorganisms in the processing environment, they are not recommended for use in the low-moisture food processing environment (Du et al., 2007). During the manufacture of peanut butter products and cleaning/sanitation of peanut butter processing equipment, the environment should be kept as free from moisture as possible. The use of water during cleaning can allow the water activity to reach levels that promote the growth of microorganisms (Beuchat et al., 2013; Codex Alimentarius, 1979). Dry sanitation procedures are used in processing environments where water must not be used, but current methods may not be appropriate for all dry cleaning situations (Beuchat et al., 2013; International Life Science Institute Europe, 2011). Currently, nut butter producers frequently push through fresh product or internal pipeline cleaning devices to remove unwanted product or clean their piping, and do not have validated chemical methods to sanitize their processing equipment. The lack of effective sanitation procedures could lead to microbial cross-contamination (Evans et al., 1996; Grocery Manufacturers Association, 2009b; Kusumaningrum et al., 2003), as seen in the 2012 *Salmonella* outbreak in Sunland, Inc. products (Centers for Disease Control and Prevention, 2012; U. S. Food and Drug Administration, 2012).

Cleaning and sanitation trials were conducted on pilot-scale stainless steel nut butter pumping equipment designed to mimic optimal processing conditions currently used in the nut butter industry (Codex Alimentarius, 2008).

Three independent cleaning/sanitizing trials were conducted for each sanitation treatment. Approximately 63.5 kg of commercial creamy peanut butter was heated in the steam jacketed tank 2 (T2). Once the temperature of the peanut butter reached ~60 °C, 800 g of inoculated peanut butter was added to T2. Surface grown cultures (sessile) were used as studies have shown their thermal resistance follows a linear trend (Keller et al., 2012). Water activity samples were collected immediately before inoculation and after 15 min mixing. The water activities of the peanut butter before and after inoculation were 0.200 ± 0.045 and 0.203 ± 0.052, respectively. It was concluded that addition of the inoculum did not have a significant effect ($n = 6$; $p > 0.05$) on the water activity of the peanut butter.

A temperature range of 60–65 °C was chosen as the inoculation and processing temperature for the peanut butter. In this temperature range the peanut butter remained in a molten state that flowed freely through the piping. In addition, previous research has shown that *Salmonella* serovars are thermally stable at temperatures under ~80 °C in peanut butter (Keller et al., 2012; Ma et al., 2009; Shachar and Yaron, 2006). Holding the peanut butter between 60 and 65 °C allowed the inoculated peanut butter to fully contaminate the system and ensured any microbial reduction was not due to thermal death during the contamination process. The final microbial count of the peanut butter used to contaminate the equipment was 7.3–7.4 log CFU/g (Table 2).

Table 2
Microbial counts in the product sample at various time points throughout the peanut butter inoculation. Samples were plated onto tryptic soy agar with yeast extract (TSAYE) and xylose lysine deoxycholate (XLD) agar.

Product sample	Microbial survival (log CFU/g) of product[a]	
	TSAYE	XLD
Inoculated peanut butter	7.4 ± 0.4 A	7.3 ± 0.4 a
Initial oil flush	6.3 ± 0.5 B	6.5 ± 1.3 b
Hot oil ($t = 0$ min)	4.5 ± 1.0 C	4.5 ± 1.0 c
Hot oil ($t = 30$ min)	4.0 ± 0.3 C	3.7 ± 0.5 c
Hot oil ($t = 60$ min)	3.6 ± 0.4 C	3.6 ± 0.9 c
Hot oil ($t = 90$ min)	3.6 ± 0.8 C	3.4 ± 1.4 c
Hot oil ($t = 120$ min)	3.2 ± 1.2 C	3.3 ± 1.6 c

[a] Different capital letters indicate significant differences ($p < 0.05$) between sampling points measured on TSAYE media; different lowercase letters indicate significant differences ($p < 0.05$) between sampling points measured on XLD media.

Although homogeneous contamination of the processing equipment was desired, it is noted that in many foods, especially low-moisture foods, distribution of pathogens may not be homogeneous. Environmental samples were taken at areas of the equipment that came into contact with the inoculated peanut butter product and on an area known not to come into contact with the contaminated peanut butter (Table 1). The environmental swabs were taken from different locations in the peanut butter processing line to determine the background contamination remaining on the equipment, and to determine the efficacy of the cleaning/sanitation of the hot oil and isopropanol-based treatments (Figs. 2 and 3).

In addition to environmental swabs, samples were taken of inoculated peanut butter, cold oil flush, and periodically of the hot oil circulated throughout the system (Table 2). Triplicate samples were obtained for each product at each sampling point. Following the removal of inoculated peanut butter from the system, the initial cold oil flush provided ~1-log CFU/mL reduction of *Salmonella* than initially present in the peanut butter present in the system. After the initial oil flush was drained, the hot oil treatment provided a second immediate reduction from the initial microbial load. Both reductions with oil could be attributed to a simple dilution effect. During the 2 h hot oil treatment, the temperature of the oil ranged from 86 °C to 90 °C (measured at $t = 0$ and 120 min, respectively). Heat was applied to the system through a steam-jacketed insulated main tank. Circulatory piping was not heated. Therefore, the oil in portions of the equipment that were not heated and/or insulated may have been at a lower temperature than oil in the heated tank. This is not atypical of what would be found in the industry. Although there was variability in oil temperature for the duration of the hot oil treatment, microbial levels enumerated from all oil samples collected during the two hour hot oil circulation were not statistically different ($p > 0.05$; Table 2), indicating no significant thermal inactivation occurred during hot oil treatment. This was not unexpected based on thermal kinetics found in the literature related to thermal destruction of *Salmonella* in this temperature range in low-moisture environments. *Salmonella* inoculated into peanut butter is resistant to heat, with the greatest inactivation occurring within the first 10–20 min of heat treatment (Ma et al., 2009; Shachar and Yaron, 2006). The same authors observed a maximum decrease of 3.2 log CFU/g at 90 °C held for 50 min, for a cocktail of *Salmonella* Agona, *S.* Enteritidis, and *S.* Typhimurium.

The lack of a reduction in *Salmonella* counts in the hot oil throughout the hot oil treatment is also reflected in the environmental swab results after hot oil treatment (Figs. 2 and 3). Total microbial populations are clearly higher after hot oil treatment indicating the failure of hot oil to reduce populations to pre-contamination levels (data not shown). This is again obvious in presumptive *Salmonella* populations (Fig. 2).

Since the results of all three trials of hot oil treatments failed to remove *Salmonella* from the peanut butter processing equipment or provide a reasonable reduction in *Salmonella* populations, using an

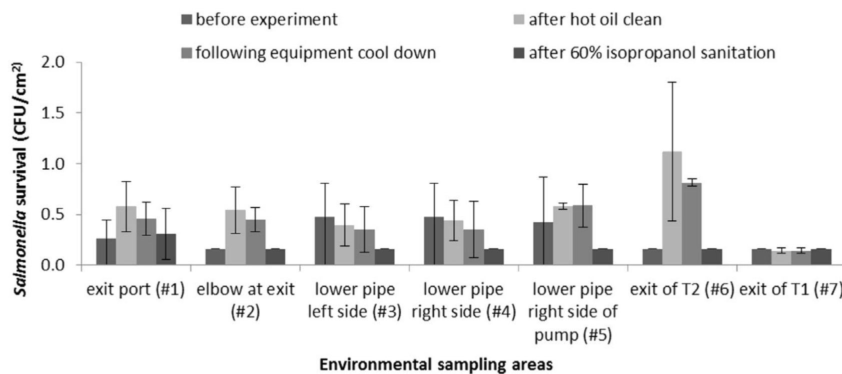

Fig. 2. *Salmonella* populations remaining on the peanut butter equipment at seven different locations as tested with environmental swabs plated onto xylose lysine deoxycholate (XLD) agar with a limit of detection of 0.16 log CFU/cm^2. Samples were taken before the start of a new trial, after the hot oil treatment, after system cool down, and after the isopropanol treatment.

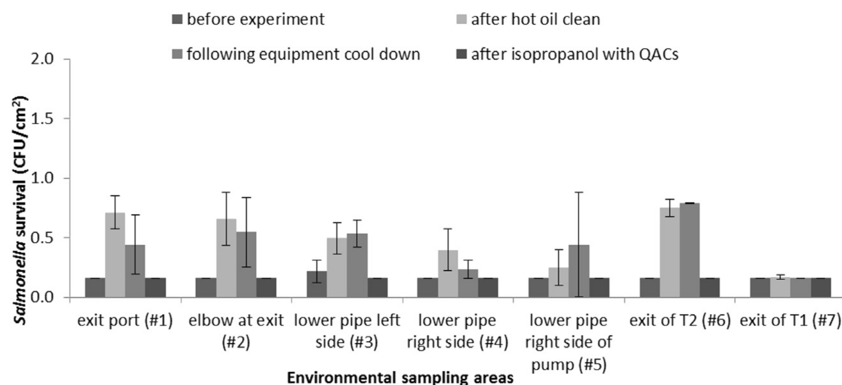

Fig. 3. *Salmonella* populations remaining on the peanut butter equipment at seven different locations as tested with environmental swabs plated onto xylose lysine deoxycholate (XLD) agar with a limit of detection of 0.16 log CFU/cm^2. Samples were taken before the start of a new trial, after the hot oil treatment, after system cool down, and after the isopropanol with added quaternary ammonium compounds treatment.

oil treatment as a sanitation step would unlikely provide appropriate levels of decontamination. However, no visible peanut butter residual was observed following the hot oil treatment.

It is critical that such sanitizing is undertaken in a manner that avoids the creation of a further safety risk. Consequently, all processing equipment was allowed to return to ambient temperatures prior to the isopropanol based sanitation procedure. Equipment was allowed to cool for 24 h prior to the use of isopropanol to ensure treatment below the flash point of isopropanol (40 °C).

Following circulation of 60% isopropanol at ambient temperature for 1 h, low levels of microbial contamination were detected on the processing equipment after plating on TSAYE. A number of colonies on TSAYE were tested using the Vitek microbial identification system, but none were found to be *Salmonella*. In addition, after isopropanol treatment there were no presumptive *Salmonella* detected in environmental swabs when plated onto XLD (detection level 0.16 log CFU/cm^2) (Fig. 2). Similar results were observed for sanitizing treatment using isopropanol with added quaternary ammonium compounds as were found for the 60% isopropanol treatment (Fig. 3). Less than 1.15 CFU/cm^2 were enumerated on TSAYE from equipment surfaces following the sanitation step, but again no *Salmonella* were detected after treatment (Fig. 3).

Du et al. (2007), also investigated the use of isopropanol-based quaternary ammonium sanitizer on the treatment of almond dust inoculated stainless steel surfaces, and found a reduction in aerobic plate counts of ~3 log CFU/g. Further studies on the use of isopropanol-based quaternary ammonium sanitizers found that they were more effective than aqueous quaternary ammonium sanitizers (Du et al., 2010). The same authors also showed similar reductions in *Salmonella* populations to results to those found here. In the previous study both isopropanol and isopropanol-based quaternary ammonium sanitizer were able to reduce *Salmonella* counts from an initial contamination level of 5.2 log CFU/g to below 1.3 log CFU/g in almond huller–sheller dust. In our studies *Salmonella* reductions were found to be as great as 4–6 log CFU/cm^2 depending on the initial contamination (Figs. 2 and 3). The use of 60% isopropanol or 59% isopropanol with 0.02% added quaternary ammonium compounds aided in the sanitation of the pilot-scale nut butter pumping equipment.

4. Conclusions

Sanitation guidelines are lacking for facilities where the use of water-based cleaning and sanitation procedures are not desirable. In this study, we have tested the use of hot oil and isopropanol sanitizers to decontaminate a simulated peanut butter processing system. Unfortunately, hot oil did not result in any appreciable reduction in *Salmonella* levels other than what would be expected through a dilution effect. However, hot oil was effective in cleaning/removing peanut butter from the processing equipment. A cleaning step is critical prior to the application of a chemical sanitizer, particularly in the presence of high-fat foods such as peanut butter, which may confer stability to the bacterial cells. Following removal of peanut butter, both 60% isopropanol and an isopropanol sanitizer containing quaternary ammonium compounds, examined independently, were highly effective with respect to reduction of *Salmonella* in the system. Both isopropanol-based sanitizers were able to effectively reduce *Salmonella* contamination by as much as 5-log CFU/g, to below detection limits.

Typical industry recommendations with respect to good manufacturing practices call for effective cleaning and sanitation at regularly scheduled intervals (Codex Alimentarius, 1979; Grocery Manufacturers Association, 2009a). A two-step, hot oil clean followed by isopropanol-based sanitizer as outlined here may provide such a procedure for an environment frequently deficient in documented effective cleaning and sanitation regiments. In addition, implementation of such procedures could allow for effective lot separation, thus reducing the risk of recalls should contamination occur and can provide an effective means for reducing or eliminating *Salmonella* contamination of nut butter processing equipment.

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