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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.

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 multi-state 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