EVALUATION OF A SANITIZING SYSTEM USING ISOPROPYL ALCOHOL QUATERNARY AMMONIUM FORMULA AND CARBON DIOXIDE FOR DRY-PROCESSING ENVIRONMENTS

by

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Abstract

Dry-processing environments are particularly challenging to clean and sanitize because water introduced into systems not designed for wet cleaning can favor growth and establishment of pathogenic microorganisms such as *Salmonella*. The objective was to determine the efficacy of isopropyl alcohol quaternary ammonium (IPAQuat) formula and carbon dioxide (CO_2) sanitizer system for eliminating *Enterococcus faecium* and *Salmonella* on food contact surfaces. Coupons of stainless steel and conveyor belting material used in dry-processing environments were spot-inoculated in the center of 5 × 5 cm coupons with approximately 7.0 log CFU/ml of *E. faecium* and up to 10 log CFU/ml of a six-serotype composite of *Salmonella* and subjected to IPAQuat-CO_2 sanitation treatments using exposure times of 30 s, 1 or 5 min. After sanitation treatments, wet coupons were swabbed for post-treatment survivors. Preliminary experiments included coupons which were soiled with a flour and water solution prior to inoculation and subsequent sanitation treatments. For the main study, inoculated surfaces were soiled with a breadcrumb flour blend and allowed to sit on the lab bench for a minimum of 16 h before sanitation. Preliminary results showed that IPAQuat-CO_2 sanitizing system was effective in reducing approximately 3.0 logs of *E. faecium* and *Salmonella* from clean and soiled surfaces after 1 min exposure but higher initial inoculum levels were needed to demonstrate >5 log reductions. For the main study, pre-treatment *Salmonella* populations were approximately 7.0 log CFU/25 cm^2 and post-treatment survivors were 1.3, < 0.7 (detection limit), and < 0.7 log CFU/25 cm^2 after 30 s, 1 or 5 min sanitizer exposures, respectively, for both clean and soiled surfaces. Treatment with IPAQuat-CO_2 sanitation system using 30 s sanitizer exposures resulted in 5.7 log CFU/25 cm^2 reductions whereas, greater than 6.0 log CFU/25 cm^2 reductions were observed for sanitizer exposures of 1 and 5 min. The IPAQuat-CO_2 sanitation system reduced 6 logs CFU/25 cm^2 of *Salmonella* with sanitizer exposure times of at least 1 min. The IPAQuat-CO_2 system would, therefore, be an effective sanitation system to eliminate potential contamination from *Salmonella* on food contact surfaces and have application in facilities that process dry ingredients or low-moisture products.

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Dedication

This thesis is dedicated to my Mom. She always stood beside me and was the strongest person I have ever known. She is gone now but never forgotten. It is also dedicated to my children, Erin and Ryan, who I wish to inspire through my perseverance and efforts. It is also dedicated to my husband, Tom, who supported me in so many ways throughout this journey.

Chapter 1 - Introduction

Cleaning and sanitation operations directly impact the safety of manufactured foods. Effective cleaning and sanitation protocols are critical to food plant hygienic conditions to prevent the buildup of adsorbed organic material, and to control the presence of foodborne pathogens in the processing environment (Boyd and others 2001). Many food processing facilities utilize detergents and sanitizers that are mixed with water to clean equipment. But, wet cleaning procedures in facilities that process dry products such as powered infant formulas, dry milk products, dry pet foods, seasonings or flavor enhancers is not always appropriate. Excess humidity from wet cleaning may alter a food plant environment. Cleaning in processing environments generally follows the rule of thumb "where equipment is dry, clean it dry; where equipment is wet, clean it wet" (Umland 2003).

Cleaning and sanitation in dry-processing environments are particularly challenging because water introduced into systems not designed for wet cleaning can favor growth and establishment of pathogenic microorganisms such as *Salmonella* (GMA 2009). Microbial niche environments can develop in cracks, crevices, pits, holes, and junctions that have accumulated food, dust, debris, and water. These areas may be hard to inspect, clean and/or sanitize, and can therefore protect microorganisms from being destroyed (Pouch Downes and Ito 2001; GMA 2009; Umland 2003). Organic material absorbed onto equipment changes the surface properties and wettability, affects strength of adhesion, and acts as a nutrient source and attachment site for microorganisms (Baier 1980; Boyd and others 2001; Schneider 1997).

The cleaning methods and tools used to "dry clean" equipment and environments are typically limited to sweeping, scraping, vacuuming, and wiping with cloths. Compressed air, brushing, and blasting with carbon dioxide (CO_2), sand or bicarbonate soda are also dry cleaning methods. Blasting technology, however, requires a secondary clean-up, and wiping with cloths has limited application and can only be useful for small areas. Problems associated with sweeping and scraping are aerosolized dust and debris which may lead to cross-contamination. It has been shown that compressed air, if strong enough, can send a small clog to parallel processing lines and be a potential source of cross-contamination. The usage of compressed air

should therefore, be highly controlled during equipment cleaning (Jackson and others 2007; Roder and others 2010).

Aerosolized dust and debris and inadequate sanitation in dry-processing environments have been linked to several multistate outbreaks of salmonellosis resulting from cross-contamination. Airborne particles of contaminated nonfat dry milk were assumed to have infected two workers from the sifting and bagging operation during an interstate outbreak of *Salmonella* Newbrunswick in 1965 and early 1966. The organism was also isolated from the air filter of the spray-dryer (Collins and others 1968). Dust and debris in the environment and inadequate sanitation of the spray-dryer and surrounding equipment could potentially have led to contamination of products.

Spray-dryers were also cited as a source of contamination in the manufacturing plant environment for powdered infant formula (PIF). Powdered infant formula has the potential to be intrinsically contaminated with organisms such as *Salmonella enterica* and *Cronobacter sakazakii* (formerly *Enterobacter sakazakii*). Dust and debris and inadequate sanitation in the production environment could potentially lead to contamination of new lots of product. Outbreaks of salmonellosis from PIF have resulted from low levels of salmonellae (Cahill and others 2008; GMA 2009; Podolak and others 2010).

Contaminated dry pet food was the cause of a multistate prolonged outbreak of *Salmonella* Schwarzengrund infections involving at least 79 human cases, 48% of which were children less than two years old (Barton and others 2010). The responsible plant was required to shut down for five months to perform cleaning and disinfection (CDC 2008). The outbreak spanned a period of three years, involved a recall of more than 23,000 tons of product and 105 brands of dry dog and cat food, and eventually the responsible plant closed. This was the first time dry pet food was reported as the cause for *Salmonella* contamination. Since this outbreak, several recalls and contaminations with multiple *Salmonella* serotypes have also been associated with a number of different contaminated pet treats and foods from multiple plants (Barton and others 2010).

Besides pet foods, pellet feeds became cross-contaminated with *Salmonella* from contaminated animal and poultry feed dust and debris present on manufacturing equipment after the heat processing step. Pellet coolers pull in large volumes of air (ca. 5,000 cfm) and contaminated dust pulled into coolers increases the chances for cross-contamination and was

found to be a major source of *Salmonella* contamination. Condensate and moisture in coolers favored pathogen growth (Jones and Richardson 2004).

Salmonella Wansdworth, a rare serotype of *Salmonella*, was reported as the source of infections in 60 persons over 19 states from March to June, 2007. No deaths were attributed to this infection; however, 10% of the patients were hospitalized and a large percent (77%) developed bloody diarrhea. Children, ages ranging from 10 months to 3 years, represented 90% of the cases. A puffed rice snack with a vegetable coating was linked to the contamination and was isolated from sealed bags of product. *Salmonella* Typhimurium was also isolated from a sealed bag of puffed rice snack during the *Salmonella* Wandsworth investigation. The recall was expanded to include other products produced by the manufacturer because of common ingredients shared with the implicated puffed rice snack (CDC 2007b; FDA 2007). Processing with contaminated ingredients could potentially lead to new products becoming cross-contaminated if cleaning and sanitation procedures between operations are not effective in eliminating the pathogenic microorganism from the processing equipment.

In 1998, a multistate infection with *Salmonella* Agona affected 209 individuals with 47 of them requiring hospitalization. The outbreak was linked by pulsed-field gel electrophoresis (PFGE) testing to a toasted oats cereal. This was the first *Salmonella* outbreak associated with a commercial cereal produced in the United States (U.S.). Because the plant manufactured multiple brands on the same manufacturing line, a voluntary recall of multiple brands ensued. Ten years later (in 2008), the same company issued a recall for two varieties of dry cereal produced over a 12 month span because they were responsible for salmonellosis which had infected at least 28 persons from 15 states. According to a statement issued by the CDC, the Minnesota Department of Health had confirmed the *Salmonella* Agona isolate obtained from the plant was linked by PFGE testing to isolates obtained from sickened individuals. According to federal and state public health officials, this was the same strain of *Salmonella* Agona that caused the outbreak linked to toasted oats cereals in 1998 (CDC 2008; CIDRAP 2008). Since *Salmonella* is reported to be able to survive for long periods in dry environments (Kusumaningrum and others 2003) and is resistant to desiccation; it is suspected that the isolate of *Salmonella* Agona may have resided in some location within the plant over the ten year period (CDC 1998; Podolak and others 2010). Any water introduced into the plant from wet cleaning

procedures (without adequate drying) could have favored growth of *Salmonella* and cross-contamination to new products.

Inadequate cleaning and sanitation operations were cited by U.S. Food and Drug Administration (FDA) representatives after visiting a dry milk producer located in Plainview, MN. Inspectional observations cited that cleaning and sanitizing operations were not adequate to protect food, food-contact surfaces, and food-packaging materials against contamination. Foamers, which can atomize microorganisms, were used to clean and sanitize equipment, floors, walls, and ceilings. After cleaning and sanitation, these areas were air-dried resulting in pools of standing water, even in dry areas such as the dry-blending room (FDA 2009a). In 2009, the dry milk producer located in Plainview, MN, voluntarily recalled a variety of products manufactured over a two year span because they were suspected to be contaminated with *Salmonella*. Several different *Salmonella* serotypes were identified from environmental samples collected from the dry milk plant.

Significant cleaning and sanitation issues were also cited by FDA officials during investigations associated with a number of recalls of hydrolyzed vegetable protein (HVP) contaminated with *Salmonella* Tennessee in 2010. Specifically, standing liquid was observed in an area where dry paste products were manufactured; and environmental samples collected from adjacent locations were positive for *Salmonella* (FDA 2010; FDA 2011a). As a flavor enhancer, HVP is an ingredient used in a wide variety of processed food products and consigned to many food processors. Products containing HVP may receive a heat treatment (soups, sauces, gravies, hot dogs, frozen dinners, and chili) or HVP may be added to foods which will be consumed without a heat treatment (seasoned snack foods, dips, and dressings). Because some foods may be consumed without a heat treatment (seasoned snack foods, dips and dressings), the presence of *Salmonella*, even in low numbers, may result in individuals becoming sick from salmonellosis (GMA 2009; Podolak and others 2010).

These examples highlight the importance of proper cleaning and sanitation in dry-processing facilities. Controlling excess moisture in dry-processing environments is essential to prevent *Salmonella* growth. Excess moisture would favor growth of the pathogen and pose a significant risk for product contamination; whereas, lack of moisture would inhibit growth of *Salmonella*. Poor sanitation practices are among the factors that have been linked to cross-contamination of low-moisture foods with *Salmonella* (Podolak and others 2010). The dry milk

producer from Minnesota sold their products to the industry and those products were incorporated into other products. Many of the final products had not been exposed to a heat treatment to inactivate *Salmonella*. Major recalls ensued involving many product categories: cakes, candies, drink mixes, instant nonfat dry milk, oatmeal, sauce mixes, topping and yogurt (286 entries in all) (FDA 2009b; FDA 2009c). These recalls highlight the complexities involved with the distribution of contaminated ingredients and illustrate the interconnectedness of the food system; one tainted ingredient can affect dozens of companies (USA Today 2009). Besides the economic losses from recalls associated with products contaminated with *Salmonella*, infections associated with *Salmonella* are reported to result in an estimate $365 million in direct medical costs annually (CDC 2010b).

Growth inhibition of *Salmonella* has been reported for water activity values less than 0.94. Low-moisture foods typically contain less than 25% moisture and have water activity values less than 0.60 (Jay and others 2005). Although *Salmonella* cells cannot grow in dry goods, they are reported to be resistant to desiccation and able to survive for a long time under dry conditions (Podolak and others 2010; Kusumaningrum and others 2003). Once water becomes available, the risk of product becoming contaminated from *Salmonella* growth increases. During the last decade, a number of outbreaks of salmonellosis impacting large numbers of individuals, and resulting in several deaths have been associated with low-moisture ready-to-eat products such as powdered infant formula, raw almonds, dry breakfast cereals, dry seasonings, dried coconut, infant cereals, peanut butter, and children's snacks made of puffed rice and corn with a vegetable seasoning. A number of factors have been linked to *Salmonella* contamination of low-moisture foods including poor sanitation practices (GMA 2009; Podolak and others 2010).

Studies conducted by Du and others (2010) investigated the use of an isopropyl alcohol-based quaternary ammonium sanitizer (IPAQuat) for reducing *Salmonella* in almond dust. The IPAQuat formula (Alpet D2; Best Sanitizers Inc., Penn Valley, Calif., U.S.A.) was obtained premixed (200 ppm quat, 58.6% isopropyl alcohol). Dust is difficult to control and a major concern in almond hulling and shelling facilities. Du and others (2010) reported up to 7 log CFU/g reductions in *Salmonella* when inoculated almond dust was treated with IPAQuat formula. They concluded that even in the presence of high levels of organic material, IPAQuat formula was an effective sanitizer.

Studies using dust inoculated with *Salmonella* Enteritidis PT 30 showed significant growth of the organism when dust was wetted using low levels of water. When concentrations of ≥50% isopropyl alcohol (IPA) alone were added to inoculated dust, an immediate reduction in *Salmonella* was observed. Even in lower concentrations of IPA (10%), *Salmonella* populations in inoculated dust declined over time (Du and others 2010). This research suggests that alcohol-based sanitizers may be appropriate for in-season hulling and shelling facility sanitation programs (Du and others 2010).

Microorganisms attached to inert surfaces are less susceptible to the effects of cleaners and chemical sanitizers than their free-living counterparts; and, sanitizer efficacy studies should involve testing adherent cells (Frank and others 1997). The ability of *Salmonella* to form biofilms and survive for long periods on dry surfaces has been reported and becomes an important factor in foodborne infections from cross-contamination (Iibuchi and others 2010; Kusumaningrum and others 2003). Salmonellae are known to survive hostile environments through biofilm formation. The growth and survival of organisms on stainless steel, commonly used in the construction of processing equipment have been widely studied (Driessen and others 1984; Lewis and Gilmour 1987; Boyd and others 2000; Podolak and others 2010). Based on available literature, however, it is unclear if salmonellae form biofilms in low-moisture environments.

A sanitizing system which utilizes carbon dioxide (CO_2) and an isopropyl alcohol quaternary ammonium (IPAQuat) formula is commercially available and could have potential for reducing *Salmonella* on food contact surfaces. The sanitizing system's technology makes it possible to sanitize without adding water to processing environments. Carbon dioxide is utilized as a propellant or carrier to deliver a spray of IPAQuat formula. The process displaces oxygen with the rapidly expanding carbon dioxide gas and the formula is rendered non-flammable. The spray of formula enables penetration into cracks, crevices, pits, holes, and junctions that can be niche environments for pathogenic bacteria. These areas are typically hard to inspect, clean and/or sanitize, and can therefore protect microorganisms from being destroyed.

The sanitizing system's active IPAQuat formula contains 58.6% isopropyl alcohol and a surfactant or low concentration of quaternary ammonium compound (200 ppm) which leaves a residual antimicrobial film and sanitizing effect after the alcohol evaporates (D2; 1 Priority Biocidal, Fort Worth,TX; Best Sanitizers, Penn Valley, CA). Isopropyl alcohol and quaternary

ammonium compound are both known to be effective sanitizing agents (McDonnell and Russell 1999; Schmidt 2009). Two manufacturers of such systems (1 Priority Biocidal, Fort Worth, TX and Biomist, Inc., Wheeling, IL) provide this technology. If this technology and tested systems prove to be effective, they could have sanitation applications in facilities that process dry ingredients or low-moisture products.

The IPAQuat-CO_2 sanitizing systems are expected to be effective for use in dry facilities because the IPAQuat formula delivered as a spray with the CO_2 system dries quickly and does not add moisture to the processing environment. Because water is not introduced into the environment, the risk of spreading pathogenic bacteria is minimized. The IPAQuat formula was tested against *Escherichia coli* and *Staphyloccus aureus* with 6.9 log CFU/carrier reduction after 1 min exposures (Bioscience 2000). The objective of this study was to evaluate the efficacy of IPAQuat-CO_2 sanitizing systems to eliminate potential *Salmonella* contamination from food contact surfaces for application in facilities that process dry ingredients or low-moisture products.

Chapter 2 - Review of Literature

Characteristics of *Salmonella*

Salmonellae are small, Gram-negative, non-spore forming rods that have been reported to cause illness for over 100 years and are widely distributed in nature (Jay and others 2005; CDC 2010a). Animals (birds, reptiles, and farm animals) and human intestinal tracts are the primary reservoirs. Salmonellae can be excreted in the feces and then transmitted to many places (Jay and others 2005). Salmonellae have been isolated from environmental sources including water, soil, insects, animal feces, raw meats, raw poultry, and raw seafood and processing surfaces in factories and kitchens (FDA 2011b).

Multiple drug resistance is an emerging problem among *Salmonella* serotypes. Previously accepted drugs known to aid and treat patients with salmonellosis are no longer useful because the organism has adapted, rendering the antibiotics as ineffective. In a survey conducted in 2003, *S.* Typhimurium isolates were resistant to multiple antimicrobial drugs (45% resistant to one or more drugs and 26% of phage type DT104 had a five drug resistant pattern). *Salmonella* Newport has also emerged as a multidrug-resistant pathogen. In 2003, 21% (46 out of 222) of serotype *Salmonella* Newport isolates were resistant to 17 antimicrobial agents including those intended for extended spectrum (CDC 2005).

Taxonomy

There are 2,400 or more *Salmonella* serotypes. Latest changes to the taxonomy of *Salmonella* have classified them into two species, *S. enterica* and *S. bongori* with the serotypes being divided into groups and subspecies. Most groups or subspecies fall into the S. *enterica* species. *Salmonella* serotypes are not treated as individual species as was a long-standing practice. These changes were made based on DNA-DNA hybridization and electrophoretic enzyme characterizations of the salmonellae. Changes to the classifications have resulted in changes in the nomenclature of salmonellae. *Salmonella* Typhimurium which falls into the *Salmonella enterica* species should be written as *S. enterica* serovar Typhimurium or *Salmonella* Typhimurium (Jay and others 2005).

For epidemiological purposes, salmonellae are placed into three groups. The group which causes typhoid and paratyphoid fevers are the most severe and infect humans only. The host-adapted serotypes contain some that may be contracted from foods, and unadapted serotypes include most of the foodborne serotypes. The unadapted serotypes have no host preference meaning they can infect both animals and humans (Jay and others 2005).

Factors for growth and survival

The pH for optimum *Salmonella* growth is around neutral, specifically 6.6 to 8.2. Values above 9.0 are considered bactericidal. Minimum growth pH has been reported to be 4.05 for products containing citric acid. However, several serotypes of *Salmonella enterica* were reported to remain viable for more than 30 days in juice samples at pH 3.9 when juice samples were held at refrigeration temperatures (Mazzotta 2001). The lowest temperatures reported for growth are 5.3°C (*S.* Heidelberg) and 6.2°C (*S.* Typhimurium). Several investigators have reported 45°C as the upper limit for growth (Jay and others 2005).

Growth inhibition of *Salmonella* has been reported for water activity (a_w) values less than 0.94 in neutral pH. Low-moisture foods typically contain less than 25% moisture and have water activity values less than 0.60 (Jay and others 2005). Higher levels of available moisture (a_w) are needed for growth as the pH decreases. Salmonellae cannot tolerate high levels of salt and brines above 9% are reported bactericidal. *Salmonella* cells were reported to be protected in dried products containing 36% sucrose; survival was increased substantially (79 times). Sucrose was said to protect cells more than fructose, glycerol, and sorbitol (Goepfert and others 1970; Podolak and others 2010).

Salmonella are readily destroyed at milk pasteurization temperatures (high temperature-short time 72ºC for 15 s). *Salmonella* are more heat sensitive in the growth phase as opposed to the stationary phase. *Salmonella* Senftenberg is reported to be 30 times more heat resistant than *S.* Typhimurium but the latter is more resistant to dry heat (Jay and others 2005). Goepfert and others (1970) found that heat resistance of eight strains of *Salmonella* increased as the water activity of the heating medium was reduced. Although they could not directly correlate water activity and heat resistance, all strains studied were more resistant to heat as the environment became drier (Goepfert and others 1970). It is also reported that salmonellae can survive temperatures as high as 90°C for 50 min and persist for a long time in a high-fat, low water

activity environment (CDC 2009; Mattick and others 2000; Podolak and others 2010; Janning and others 1994).

Salmonella has been reported to survive for a long time under dry conditions (water activity less than 0.94). Salmonellae desiccated on anhydrous silica gel were studied and reported to be stable under dried conditions (a_w of 0.2 at 22ºC); a 1-log reduction in numbers was reported after 8 months to almost 4 years of storage. *Salmonella* Choleraesuis survived in dried feces for more than a year, *Salmonella* Typhimurium and *Salmonella* Thompson survived in skim milk powder for more than 8 weeks and nontyphoid *Salmonella* survived on paper disks for 24 months (Iibuchi and others 2010). *Salmonella* Enteritidis has been reported to remain viable on dry stainless steel surfaces and was recovered, as shown in Table 2.1 (Kusumaningrum and others 2003), for at least 96 h or 4 days when the contamination level was high (10^5 CFU/cm^2). When the level of contamination was moderate (10^3 CFU/cm^2), the level of surviving cells decreased below the detection limit (log cell numbers = 0.62 CFU/100 cm^2) within 24 h. Low levels of contamination (10 CFU/cm^2) were below the detection limit after 1 h (Kusumaningrum and others 2003; Podolak and others 2010). Five separate contact plates shown in Table 2.2 (Kusumaningrum and others 2003) demonstrate that *S.* Enteritidis was present and recoverable from the same spot multiple times; which is important when considering potential risks for contamination of food from food contact surfaces.

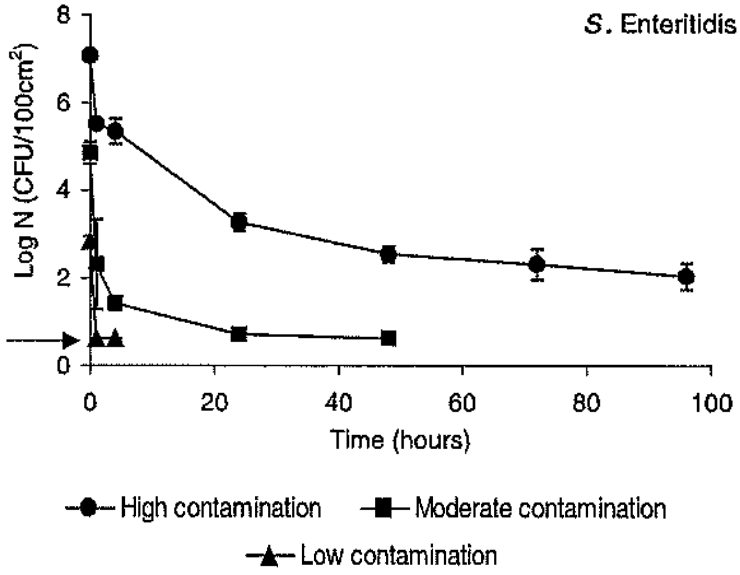

Figure 2.1 *Survival of* Salmonella *Enteritidis on stainless steel at room temperature (22-25ºC, 40-45% RH) at different contamination levels (Kusumaningrum and others 2003)*

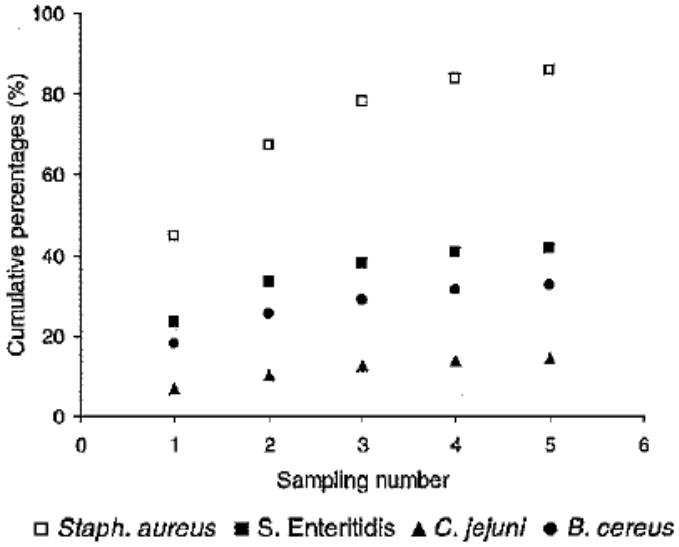

Figure 2.2 *Cumulative percentages of* Salmonella *Enteritidis and other microorganisms recovered from stainless steel surfaces on the same spot using five consecutive contact plates (Kusumaningrum and others 2003)*

Biofilm formation is believed to aid bacterial survival under dry conditions. *Salmonella* can form biofilms at solid-liquid and air-liquid interfaces. In experiments conducted by Iibuchi

and others (2010), *Salmonella* strains that produced relatively higher levels of biofilms survived desiccation on polypropylene discs much longer than isolates with low biofilm activity. The biofilm producing strains formed an exopolymeric matrix observed using scanning electron microscopy (SEM) and crystal violet staining. The findings suggest that the exopolymeric matrix acts as a barrier which maintains moisture within the cells and also can protect the cells from external stresses such as cleaning chemicals. Human isolates of *Salmonella* are reported to produce large amounts of extracellular material responsible for biofilm formation. These results suggest that *Salmonella* strains capable of producing biofilms are a greater risk to contaminating foods because they have the capability to survive longer in the environment (Iibuchi and others 2010). A strain of *Salmonella* Agona is believed to have persisted in a manufacturing facility over a 10 year time period. The same strain was responsible for two outbreaks of salmonellosis from the consumption of two different dry cereals manufactured in the same facility a decade apart (GMA 2009).

Salmonellosis

Salmonellosis is a foodborne gastroenteritis caused by ingesting foods containing appropriate strains and numbers of organisms from the genus *Salmonella* (Jay and others 2005). The infective dose can be as few as 15 to 20 cells dependent on the age and health of the host as well as the strain of *Salmonella* (FDA 2011b). Persons infected with *Salmonella* can experience diarrhea, fever, and abdominal cramping. Infections are confirmed with positive stool samples. The onset of illness for most persons is 12 to 72 h after ingestion. Most individuals recover within 4 to 7 days without treatment; however, some patients with severe diarrhea may need hospitalization. In this case, *Salmonella* infection has spread from the intestines to the blood stream and other body sites. Severe illnesses need to be treated with antibiotics or death may result. Persons most likely to develop severe illnesses are elderly, very young or those with weakened immune systems. Long term complications may result in arthritic conditions even for those treated with antibiotics (CDC 2010a; FDA 2011b).

Salmonellosis has been associated with the consumption of low-moisture products containing very low levels of *Salmonella* organisms. Infections may occur from ingesting foods containing less than 1 CFU/g dependent on the strain of *Salmonella*, the product, and host. Some of the products associated with salmonellosis from low numbers have been paprika, paprika-powdered potato chips, chocolate, and peanut butter. The latter two foods being high fat

supports the assumption that food composition may actually protect *Salmonella* against the acidic conditions of the stomach. Therefore, it can be concluded that low numbers of *Salmonella* can colonize the gastrointestinal tract and cause infection (GMA 2009).

Foods Associated with *Salmonella*

Eggs have frequently been linked to *Salmonella* Enteritidis (SE) infections which can be contracted from improperly cooked normal-appearing eggs (CDC 2010b). Poultry, raw milk, pork, beef, sprouts, and raw almonds have been linked to SE infections since early 2000s. Shell eggs and poultry have been identified as the most common sources for SE infections (CDC 2004). *Salmonella* Enteritidis infections have also been linked to handling reptiles. Farm animals or flocks of poultry may be contaminated with *Salmonella* through their excrement. Contamination may occur at any point during further processing: defeathering, slaughtering, chilling, and fabrication. Accidental puncturing of the intestinal lining creates substantial opportunities for cross-contamination in the slaughtering process (Buzby and others 1996).

Flour is a raw agricultural commodity which may not be treated to kill pathogens. Low levels of *Salmonella* contamination have been identified from wheat flour, and flour or flour based mixes. Because flour is purchased in bulk, one lot could contaminate multiple batches of finished product (Sosland 2011). Many cookie dough manufacturers now source heat-treated or pasteurized flour for use in production and state that food processors of ready-to-bake or ready-to-cook foods that may be consumed without heating should consider using pasteurized flour (Sosland 2011).

From 1962 to 1966 outbreaks of salmonellosis were investigated and common vehicles of infection were typically poultry products (*S*. Derby outbreak of 1963 and 1964) and new reservoirs such as cottonseed flour, dried yeast, and pet turtles were recognized (Collins and others 1968). *Salmonella* Typhimurium, the most common serotype in humans, is mostly associated with clinical samples from cattle sources and non-clinical samples from chicken sources. *Salmonella* Enteritidis and Heidelberg, the second and fourth most commonly identified isolates linked to samples; clinical and non-clinical, are related to chicken sources (CDC 2005).

Peanut butter was reported as the source of salmonellosis in Australia in 1996 and *Salmonella* Mbandaka was identified as the causative agent. Peanut butter was again reported as the source of salmonellosis from August 2006 to April 2007. Peanut butter contaminated with

Salmonella Tennessee infected at least 628 persons from 47 states in the U.S. Two different brands of peanut butter manufactured at the same plant were responsible for the infections. *Salmonella* Tennessee was isolated from opened and unopened jars of peanut butter and from environmental samples collected at the plant (CDC 2009). The U.S Food and Drug Administration (FDA) conducted an extensive inspection of the processing plant and revealed that the probable cause of *Salmonella* contamination was a leaky roof and faulty sprinkler system which moistened the peanuts or dust and allowed the growth of the organism (FDA 2009d). *Salmonella* Tennessee is rare and represented 0.1% of all reported strains from 1995-2004 (CDC 2007).

Peanuts can be contaminated at many points in the process; during growth, harvest or storage. Although peanuts undergo a heat treatment, >70°C, they may still contain salmonellae. Cross-contamination can come from raw peanuts, poorly implemented good manufacturing practices (GMP's), or from other raw ingredients used in the processing environment. This outbreak which reached 47 states demonstrated the potential for widespread illness from one contaminated product. Although no illnesses were definitively linked to use of this product in other countries, product manufactured from the plant was exported to 70 countries (CDC 2007). Similarly, raw almonds have been linked to *Salmonella* contamination. After an outbreak of salmonellosis in 2000 to 2001 associated with raw almonds, hulling and shelling facilities were required to register as food processing facilities. The processing facility subsequently needed to make significant improvements to in-season cleaning and sanitation procedures (Du and others 2007)

In January 2009, 529 persons across 43 states in the U.S. and one person from Canada were reported to be infected with *Salmonella* Typhimurium. Eight deaths potentially resulted from the infection. Lab and epidemiologic findings indicated that peanut butter and peanut paste produced at one plant located in Blakely, GA, was the source of the outbreak. These products were ingredients for many other food companies. Peanut butter crackers produced by another company were linked to the same outbreak strain. The infection and outbreak highlights the complexities involved with distribution of contaminated food ingredients. Products were distributed through various channels and involved 2,100 accounts and sub-accounts. A recall expanded to all products produced at the plant over a two year timeframe ensued. By late January, a reported 54 companies had recalled at least 431 peanut butter containing products

because they had the potential to have been contaminated with ingredients produced by the implicated plant. Peanut butter and peanut paste are common ingredients used in cookies, crackers, cereal, ice cream, pet treats, and other foods. Mass distribution can lead to nationwide outbreaks which are widely distributed. Therefore, rapid outbreak detections and investigations are important (CDC 2009).

Significance of *Salmonella* to Public Health

According to CDC, approximately 40,000 cases of salmonellosis are reported per year in the U.S. This number may be actually lower than the number of persons infected because milder cases might not be diagnosed or reported. Approximately 400 persons die each year with acute salmonellosis (CDC 2010a). Control of *Salmonella* is challenging because of its ubiquitous nature and that it can cycle between a host and environment and back into another host, e.g., from animals to soil and water and from contaminated soil and water back into other animals (Podolak and others 2010). *Salmonella* can contaminate a wide range of foods, and different serotypes tend to have different animal reservoirs and food sources, making control challenging (CDC 2010b).

According to data analyzed from The Foodborne Diseases Active Surveillance Network (FoodNet) spanning 1996 to 2005, person's ≥65 years old were the most affected and *Salmonella* remains one of the leading pathogens in U.S. fatalities (Behravish and others 2010). While the CDC reports that FoodNet 2010 data shows a downward trend in foodborne infections, rates of *Salmonella* infection were 3% higher in 2010 when compared to the period of years including 1996 to 1998 and 10% higher when comparing the period of years including 2006 to 2008 (CDC 2011a, CDC 2011b).

According to data from FoodNet, a total of 19,089 laboratory-confirmed infections, 4,247 hospitalizations, and 68 deaths were reported from FoodNet sites in 2010. This network is a collaborative partnership between CDC and state health departments located in Connecticut, Georgia, Maryland, Minnesota, New Mexico, Oregon, Tennessee, and certain counties in California, Colorado, and New York. FoodNet conducts surveillance among 15% of the U.S. population which is approximately 46 million people. Nine pathogens commonly transmitted through food are monitored by laboratory-confirmed infections. *Salmonella* infection was associated with the most deaths (29) and hospitalizations (2,290) and was the most common

infection reported (8,256 infections; 17.6 illnesses per 100,000 persons). Children aged <5 years had the highest incidence (69.5 infections per 100,000 children). Of the 7,564 (92%) *Salmonella* isolates serotyped, the most common serotype was Enteritidis (22%) which was a 76% increase for this serotype compared to 1996 to 1998. *Salmonella* Typhimurium accounted for 13% of the serotypes identified and was a 53% decrease compared to 1996 to 1998. When compared to 2006 to 2008, incidence was significantly higher for Enteritidis (36% increase) and Typhimurium did not change significantly. *Salmonella* infections are reported to result in an estimated $365 million in direct medical costs annually (CDC 2010b).

Salmonella Outbreaks Linked to Dry Food Ingredients

A foodborne outbreak is when two or more people become sick from ingesting the same contaminated food or beverage (CDC 2011c). Public health officials investigate outbreaks and use the lessons learned to prevent future occurrences. A report collated by the Grocery Manufacturers Association (GMA)(2009) identified outbreaks of salmonellosis from the consumption of ready-to-eat low-moisture products over the last several decades. The affected products have included chocolate, powdered infant formula, raw almonds, toasted oats breakfast cereals, dry seasonings, paprika-seasoned potato chips, dried coconut, infant cereals, peanut butter and products containing peanut butter, and children's snacks made of puffed rice (GMA 2009).

Nonfat dry milk was cited as the vehicle of infection during an interstate outbreak of *Salmonella* Newbrunswick in 1965 and early 1966 (Collins and others 1968). The same serotype was identified from products found on the grocery shelves and from a milk-drying plant. It was hypothesized that *S.* Newbrunswick came from one of the farms supplying raw milk and that the initial heat treatment of skim milk was not adequate leading to contamination of the finished product and the processing environment. Analysis of samples from different milk products and from the environment pointed to the instantizing process as the source of contamination.

Two workers from the sifting and bagging operation were infected with *S.* Newbrunswick and were thought to have been infected from exposure to airborne particles of contaminated milk. The organism was also isolated from the air filter of the spray-dryer. Subsequent surveys of milk-drying plants from various states led to the isolation of numerous *Salmonella* serotypes. Instant nonfat dry milk is widely distributed in the U.S. and to many under-developed countries

indicating the potential for sizable outbreaks if contaminated product is distributed (Collins and others 1968).

Powdered infant formula (PIF) is not a sterile product that has the potential to be intrinsically contaminated, and organisms of greatest concern are *Salmonella enterica* and *Cronobacter sakazakii* (formerly *Enterobacter sakazakii).* Infected infants and young children, especially those immunocompromised, are more likely to become severely ill or die due to salmonellosis. Higher incidences of salmonellosis have been diagnosed and reported for infants (8 times greater); which could be attributed to susceptibility, exposure, or the tendency for this age group to need medical care and have stool samples tested (Cahill and others 2008).

The PIF spray-dryers have been a source of contamination in the manufacturing plant environment. Distribution of salmonellae is described as sporadic or heterogeneous which makes detection of the microorganism challenging when relying on finished product testing. Outbreaks of salmonellosis from PIF have been attributed to low levels of salmonellae. It is imperative to develop measures at the manufacturing level to minimize the potential for intrinsic contamination (Cahill and others 2008).

From May to July 1993, three cases of infants infected with *Salmonella* Tennessee were linked to the consumption of contaminated powdered infant formula in Canada and the U.S. The strain was atypical because it was able to ferment lactose. The *Salmonella* serotype Tennessee was first isolated from infant stools who had consumed infant formula and later from the Minnesota plant where the product had been dried, as well as from cans of prepared formula. From November 1992 to June 1993, 48 cases of infection with *Salmonella* Tennessee were reported to CDC. In late June, 1993, FDA recalled all products spray-dried at this plant since early November, 1992 (CDC 1993).

Contaminated dry pet food was the cause of a multistate prolonged outbreak of *Salmonella* Schwarzengrund infections involving at least 79 human cases, 48% of which were children less than two years old (Barton and others 2010). Because only 3% of infections are confirmed or reported, the actual infection may have been larger. The responsible plant was required to shut down for five months to perform cleaning and disinfection (CDC 2008). The outbreak spanned a period of three years, involved a recall of more than 23,000 tons of product affecting 105 brands of dry dog and cat food and eventually the responsible plant was permanently closed. This was the first reported *Salmonella* infection from contaminated dry dog

food. Since this outbreak, several recalls and contaminations with multiple *Salmonella* serotypes have also been associated with a number of different contaminated pet treats and foods from multiple plants (Barton and others 2010). The FDA regulates pet foods, treats and supplements, and the presence of *Salmonella* indicates that these products are adulterated under the Federal Food, Drug and Cosmetic (FDC) Act (CDC 2008).

Besides pet foods, animal and poultry feed ingredients, particularly feed dust and debris, are a source of *Salmonella* contamination. Dust in manufacturing can cross-contaminate pellet feeds after the heat process (Jones and Richardson 2004). Pellet coolers pull in large volumes of air (ca. 5,000 cfm) and contaminated dust pulled into coolers increases the chances for cross-contamination. Dust in coolers was found to be a major source of *Salmonella* contamination. Condensate and moisture in coolers favored pathogen growth. *Salmonella* serotypes isolated from poultry feeds have been linked to isolates found weeks later in chicks, breeding flocks, and commercial eggs (Jones and Richardson 2004).

For a period of monitoring spanning a decade, it was reported that approximately 80% of *Salmonella* serotypes isolated from poultry feeds were the same serotypes as those found weeks later in breeding flocks and chicks. In another study, pulsed-field gel electrophoresis (PFGE) patterns were used to link the same strain of *S.* Enteritidis found in commercial eggs sold in Japan directly to the feed. A logical assumption would be that the contamination originated from the feed. Contamination in the feed was not uniformly distributed and the few cells present may have been harder to detect because they were often damaged in the processing of the feed. Accurate assessments for contamination rates were difficult (Jones and Richardson 2004).

A tree nut recall for *Salmonella* contamination was identified in 2001. In this instance, raw almonds were the source of the *Salmonella* Enteritidis infections, which occurred mostly in Canada. The contamination was traced to three California orchards linked to SE isolates of identical phage and PFGE patterns. Almonds from these orchards could not be sold as “raw” and needed to be used in processed foods only (CDC 2004). Raw nuts can be treated by several methods to mitigate the risk for bacterial contamination. However, the drying and hulling-shelling practices can lead to cross-contamination in the processing environment.

In May 2004, approximately 13 million pounds of raw almonds were recalled by the producer due to contamination with *Salmonella* Enteritidis. These almonds were distributed throughout the U.S. and Canada. Oregon’s State Public Health Laboratory originally identified a

cluster of five patients using two-enzyme PFGE. Subsequent investigations identified matching SE isolates from 29 patients in 12 states and Canada which dated back to September 2003. The outbreak continued for possibly up to a year without being detected. All state public health agencies can perform PFGE and participate in Pulse Net. Phage typing is performed by the CDC on a limited basis (CDC 2004).

Salmonella Wansdworth, a rare serotype of *Salmonella*, was reported as the source of infections in 60 persons over 19 states from March to June 2007. No deaths were attributed to this infection; however, 10% of the patients were hospitalized and a large percent (77%) developed bloody diarrhea. Children, ages ranging from 10 months to 3 years, represented 90% of the cases. A puffed rice snack with a vegetable coating was linked to the contamination and was isolated from sealed bags of product. A voluntary recall was ordered by the manufacturer. *Salmonella* Typhimurium was also isolated from a sealed bag of puffed rice snack during the *Salmonella* Wandsworth investigation; and the recall was expanded to include other products produced by the manufacturer. This recall was initiated because the other products shared common ingredients with the implicated puffed rice snack (CDC 2007b).

During April to May 1998, *Salmonella* Agona infections were reported from 11 states. This infection affected 209 individuals with 47 of them requiring hospitalization. The outbreak was linked by PFGE testing to a toasted oats cereal. This was the first *Salmonella* outbreak associated with a commercial cereal produced in the U.S. The plant that manufactured the implicated cereal produced multiple brands of plain toasted oats on the same line and had to conduct a voluntary recall of multiple brands of this product. Consumers were urged not to eat plain toasted oats manufactured by the plant until they identified the source, scope, and magnitude of the contamination (CDC 1998).

Ten years later (2008), the same company issued a recall of two varieties of dry cereal contaminated with *Salmonella* Agona. The recall involved cereals produced over a 12 month span. A statement issued by the CDC stated that the Minnesota Department of Health had confirmed that the *Salmonella* Agona isolate obtained from the plant was linked by PFGE testing to isolates obtained from infected individuals who had eaten the dry cereal (CIDRAP 2008). At least 28 persons from 15 states were infected with salmonellosis. The median age for patients was 65 years; ages ranged from 4 months to 95 years. No deaths were associated with the outbreak; however, eight hospitalizations were reported (CDC 2008; CIDRAP 2008). According

to federal and state public health officials, this was the same strain of *Salmonella* Agona that caused the outbreak linked to toasted oats cereal in 1998. It is suspected that the isolate of *Salmonella* Agona may have resided in some location within the plant over the ten year period (CIDRAP 2008). *Salmonella* are reported to be able to survive for long periods in dry environments and are resistant to desiccation (CDC 1998).

In 1998, *Salmonella* Agona was considered an uncommon serotype and represented 1.5% of human isolates reported to Public Health Laboratory Information Systems. This serotype of *Salmonella* was linked to several animal reservoirs including poultry, cattle, and pigs. The first reported case of *Salmonella* Agona contamination was in 1972 and was associated with animal feed manufactured from contaminated fishmeal. Other contaminations linked with *Salmonella* Agona have been identified from commercially produced peanut-flavored snacks and dried milk products (CDC 1998; CIDRAP 2008).

Recalls of Dry Food Ingredients Contaminated with *Salmonella*

In 2009, a dry milk producer located in Plainview, MN, voluntarily recalled a variety of products manufactured over a two year span because they were suspected to be contaminated with *Salmonella*. Several different *Salmonella* serotypes were identified from environmental samples collected from the dry milk plant. Inspectional observations made by FDA representatives cited that cleaning and sanitizing operations were not adequate to protect food, food-contact surfaces, and food-packaging materials against contamination. Foamers, which can atomize microorganisms, were used to clean and sanitize equipment, floors, walls, and ceilings. After cleaning and sanitation, these areas were air-dried resulting in pools of standing water, even in dry areas such as the dry-blending room (FDA 2009a). Controlling excess moisture in dry processing environments is critical to preventing *Salmonella* contamination of low-moisture products. Excess moisture would favor growth of the pathogen and pose a significant risk for product contamination; whereas, lack of moisture would inhibit growth of *Salmonella*. Poor sanitation practices are among the factors that have been linked to cross-contamination of low-moisture foods with *Salmonella* (Podolak and others 2010).

The Minnesota based dry milk producer sold their products to the industry and those products were incorporated into other products. Many of the final products had not been exposed to a heat treatment to inactivate *Salmonella*. Major recalls ensued involving many

product categories: cakes, candies, drink mixes, instant nonfat dry milk, oatmeal, sauce mixes, topping, and yogurt (286 entries in all) (FDA 2009b; FDA 2009c). A small investigation of a contaminated milkshake power eventually became a nationwide recall of related products associated with a dry milk manufacturer. Recalls can cascade through the food system and illustrate the interconnectedness of the system; one tainted ingredient can affect dozens of companies (USA Today 2009).

In 2010, a number of recalls were associated with *Salmonella* Tennessee isolated from hydrolyzed vegetable protein (HVP). As a flavor enhancer, HVP is used in a wide variety of processed food products. Products containing HVP may receive a heat treatment (soups, sauces, gravies, and chili) or HVP may be added to foods which will be consumed without a heat treatment (seasoned snack foods, dips, and dressings). Because these foods may be consumed without a heat treatment, the presence of *Salmonella* may result in individuals becoming sick from salmonellosis. The FDA environmental sampling process isolated *Salmonella* from the HVP manufacturing facility. Upon inspection of the facility, FDA found significant issues with the company's cleaning and sanitation procedures of the equipment. Specifically, standing liquid was observed in an area where dry paste products were manufactured and environmental samples collected from adjacent locations were positive for *Salmonella* (FDA 2010; FDA 2011a).

The FDA in taking a proactive approach to protect the nation's food supply have initiated a number of steps which includes increased sampling and environmental monitoring coupled with zoning targeting microbial niches, improved inspection and sampling techniques, and increasing the number of companies inspected including suppliers of ingredients. The FDA's outreach into the food industry led to the development of guidance documents to control *Salmonella* in low-moisture products (FDA 2010). One such document prepared by GMA and a number of food safety industry representatives representing a *Salmonella* Control Task Force (2009) reviewed current industry practices and identified specific *Salmonella* control elements such as: preventing ingress and minimizing growth, enhancing strict hygienic practices and design principles, validation and verification control measures as well as, establishing a raw materials/ ingredients control program. According to GMA, presence of *Salmonella* in low-moisture products may be attributed to processing with contaminated (raw) ingredients, not supplying an adequate thermal treatment or from post-thermal, post-processing contamination. Adding contaminated raw ingredients is a serious concern in a dry-blending operation especially

if the process does not have an inactivation step or if the ingredient is added after the thermal process (GMA 2009).

Sanitizing

Effective cleaning and sanitation protocols are critical to food plant hygienic conditions to prevent the buildup of adsorbed organic material (soil) and control the presence of foodborne pathogens in the processing environment (Boyd and others 2001). Adsorbed organic material can collect in surface cracks, change equipment surface properties and wettability, affect strength of adhesion, and act as a nutrient source and attachment site for microorganisms (Baier 1980; Boyd and others 2001; Schneider 1997).

Sanitizer efficacy studies are often conducted using planktonic cells in broth cultures. Mosteller and Bishop (1993) state that false assumptions may be made with respect to sanitizer efficacy in the processing environment if testing done in the laboratory is conducted with nonadherent bacteria. Even though sanitizers produce a 5-log reduction in a suspension test, it cannot be assumed that the sanitizer will react with the same intensity toward adherent bacteria or those with an intact glycocalyx (Mosteller and Bishop 1993); and sanitizer efficacy studies should involve testing adherent cells using conditions similar to those expected in the processing environment (Frank and Chmielewski 1997; Mosteller and Bishop 1993). Bacterial microcolonies, commonly found in nature, can adhere to a multitude of surfaces because the cells form biofilms by becoming enveloped in a glycocalyx matrix (Mosteller and Bishop 1993). The ability of microorganisms to form biofilms and survive for long periods on dry surfaces has been reported and becomes an important factor in foodborne infections from cross-contamination (Iibuchi and others 2010; Kusumaningrum and others 2003). The growth and survival of organisms on stainless steel, commonly used in the construction of processing equipment (Boyd and others 2000; Driessen and others 1984; Lewis and Gilmour 1987), have been widely studied.

Sanitation in its simplest form, i.e. rinsing and drying, was reported to be destructive to vegetative microorganisms such as *Staphylococcus aureus, Pseudomonas fluorescens,* and *Escherichia coli.* Washing or rinsing removed most organisms and exposed those left behind to unfavorable environments created from the drying process. The drying process was injurious to cells and extended drying periods (4 hours) were reported to be destructive to most vegetative

cells. Therefore, it was concluded that surviving organisms contaminating processed foods probably arise from harborages in the processing environment (Maxcy 1975).

Alcohols and quaternary ammonium compounds as sanitizers

It is generally believed that alcohols cause membrane damage and protein denaturation, which causes interference with cellular metabolism and results in cell lysis (McDonnell and Russell 1999). By altering membrane permeability, cells are more susceptible to other agents (Jay and others 2005). The antimicrobial activity of alcohols has long been relied upon and is optimal in the 60-90% range (McDonnell and Russell 1999). Somewhat affected by organic matter, alcohols leave no residues on surfaces (Wistreich and Lechtman 1976). Alcohols are widely used for hard-surface disinfection and isopropyl alcohol is considered slightly more efficacious against bacteria (McDonnell and Russell 1999).

Quaternary ammonium compounds (QACs) are positively charged cations which are attracted to negatively charged materials such as bacterial proteins; they target the integrity of the cytoplasmic membranes (lipid or protein) of bacteria. Once the QACs are absorbed into the cell wall, reactions with the cell membrane result in a disorganization followed by leakage of intracellular material, degradation of proteins and nucleic acids, and finally cell lysis. Quaternary ammonium compounds are excellent for hard-surface cleaning and have been used through the years for a variety of antiseptic and disinfectant purposes. An advantage of using QACs is the residual antimicrobial film they leave behind (McDonnell and Russell 1999; Schmidt 2009).

The IPAQuat formula (D2, Best Sanitizers Inc., Penn Valley, CA and 1 Priority Biocidal LLC, Fort Worth, TX) contains isopropyl alcohol (58.6%) and a blend of quaternary ammonium compounds at 200 ppm. IPAQuat formula was tested by independent third party lab (Microbiotest) and found to be effective against a variety of organisms including *Salmonella* using the AOAC Use-Dilution Test Method of analysis. IPAQuat formula was reported to be effective against *S*. Choleraesuis (4.56 log CFU/coupon) when exposed for 5 min at 20 ± 1°C (Hollingsworth 2003).

Cleaning and sanitation in dry-processing environments

Dry cleaning is the preferred method in a dry environment. Water introduced into a dry system and not thoroughly dried will allow bacteria to flourish, especially material which may

have collected in cracks and crevices (Umland and others 2003). Brushes used for cleaning should be food-grade, clean, color-coded, up and off the floor, inspected and sanitized frequently. Vacuuming from a central vacuum system is preferred over brushes. Portable vacuums are acceptable as long as filters and bags are kept clean. Vacuum attachments should be color-coded separating floor from food surface contact cleaning. When water is needed, the system needs to be thoroughly dried before use. Care must be taken when using high pressure-sprayers as they can move dirt to other areas. Cleaning in dry-processing generally follows the rule of thumb; "where equipment is dry, clean it dry; where equipment is wet, clean it wet" (Umland and others 2003).

Large volumes of dust can be created anywhere flour is moved or mixed. Good dust collection systems are needed. Cleaning may be as frequent as hourly or less frequent such as weekly dependent upon the system design or ingredients. Dry-processing plants can run continuously for 5 to7 days because of the nature of the process and the waste involved with starting and stopping the process. Walls, floors, and ceilings should be inspected frequently and cracks and crevices repaired. Facility inspections should include areas not easily or readily seen as well as dead spaces on equipment (Umland and others 2003).

The cleaning methods and tools used to "dry clean" equipment and environments are typically limited to vacuuming, sweeping, scraping, and wiping with cloths, using compressed air or blasting with carbon dioxide (CO_2), sand, or bicarbonate soda. The problems associated with sweeping and scraping are aerosolized dust and debris which may lead to cross-contamination. Wiping cloths have limited application and can only be useful for small areas (Jackson and others 2007). It has been shown that compressed air, if strong enough, can send a small clog to parallel processing lines and be a potential source of cross-contamination. Thus, its usage should be highly controlled during equipment cleaning (Roder and others 2010). Epidemiological and environmental investigations have suggested that cross-contamination is a major source of contamination by *Salmonella* in low water activity foods (Podolak and others 2010).

Cleaning and sanitation in dry-processing environments are particularly challenging because water introduced into the system can increase the risk of ingress or the spreading of pathogenic organisms, create niche environments for equipment that is designed to be dry cleaned, and create biofilms. A niche environment may be one that is hard to inspect, clean and/

or sanitize and therefore protects microorganisms such as *Salmonella*. These microbial niches can occur on equipment or in the environment and be attributed to cracks, pits or holes, and hard to clean junctions that have accumulated food, dust, debris, and water (GMA 2009).

Manufacturing facilities should be designed to prevent entry of *Salmonella* into high hygiene areas and minimize growth or establishment in harborage sites. Inadequate separation of wet and dry areas or movement of employees and equipment can lead to cross-contamination and entry of *Salmonella*. The application of wet-cleaning to areas designed to be dry-cleaned has been linked to *Salmonella* contamination of powdered infant formula. The establishment of *Salmonella* in the dry-processing environment is favored by the presence of water, and the collection of processing materials through inadequate cleaning protocols (CAC 2008).

Dry cleaning is important in facilities that were not designed using the more recent sanitary design principles or for older facilities where the potential for cracks and harborage sites exist and are difficult to eliminate. Dust and food residues which deposit in these sites and remain dry can increase potential microbiological problems. Once water enters these sites, microbial growth can ensue and risk contamination to the products and environment. Industry experience has shown that microbial contamination of a dusty environment may be less than a wet-cleaned environment without "visual" dust. Wet-cleaning, which introduces moisture into difficult to clean areas or into floor cracks and under equipment supports where complete drying is not achieved may lead to serious *Salmonella* problems (GMA 2009).

Environmental Protection Agency (EPA) sanitizer requirements

Sanitizers are used to reduce microorganisms from inanimate objects and surfaces to safe and acceptable levels as deemed necessary by public health codes or regulations. They can be approved for food contact and non-food contact surfaces. Rinses used for surfaces in food-processing plants which come into contact with consumable food products need to be approved as food contact sanitizers. According to the U.S. Environmental Protection Agency (EPA), sanitizing rinses for previously cleaned food-contact surfaces containing quaternary ammonium compounds must be validated for efficacy and data derived from the Association of Official Analytical Chemists (AOAC) Germicidal and Detergent Sanitizers Method. As a performance standard, results must demonstrate a 99.999% reduction in the number of microorganisms within 30 s (EPA 1979; Mosteller and Bishop 1993).

Validation Research of Different Sanitizers or Sanitizing Systems

In a study conducted at the Cleveland Veterans Affairs Medical Center, Jury and others (2010) found that application of an alcohol-based power sanitizing system to inoculated laboratory bench tops effectively reduced populations of methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus* (VRE). A reported 3 to 4 log reduction was seen when surfaces were thoroughly wet after application. Efficacy was reduced to approximately 2 logs when a lighter mist of sanitizer was applied. Out of a survey of 11 users, 10 or 91% found the system easy to use and believed it provided a thorough coverage even if surfaces were uneven and irregular. The limitations of the system were reported to be the actual application of the solution. A lighter mist might not provide a thorough coverage and not be as effective (Jury and others 2010).

Studies conducted using isopropyl alcohol to reduce *Salmonella* Enteritidis PT 30 inoculated into almond dust showed immediate reductions when concentrations of ≥50% isopropyl alcohol (IPA) were added to inoculated dust. As shown in Table 2.3 (Du and others 2010), even in lower concentrations of IPA e.g., ≥10%, *Salmonella* populations in inoculated dust declined over time (Du and others, 2010).

Table 2.1 *Survival of* Salmonella *Enteritidis PT 30 in inoculated dust D-6 treated with isopropyl alcohol at various concentrations and stored at 30 °C (Du and others 2010)*

Treatment solution [a]	Number 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 ($p < 0.05$).

A commercially available isopropyl alcohol-based quaternary ammonium sanitizer (IPAQuat) was also evaluated for its efficacy in reducing *Salmonella* Enteritidis PT 30 inoculated into almond dust (Table 2.4)(Du and others 2010). The IPAQuat formula (58.6% isopropyl alcohol, 200 ppm quat) was obtained premixed (Alpet D2; Best Sanitizers Inc., Penn Valley, Calif., U.S.A.). Dust is difficult to control and a major concern in almond hulling and shelling facilities. When 1 ml of IPAQuat formula was added to inoculated dust samples, an immediate reduction of *Salmonella* to <1.3 or 2.7 log CFU/g was reported. Both samples had <1.3 log CFU/g of *Salmonella* after 48 hours at 30ºC. *Salmonella* Enteritidis PT 30 was the isolate associated with raw almond outbreaks in 2000 to 2001 and was isolated from a processor and hulling and shelling facility months after the implicated almonds were processed. The ability of *Salmonella* to survive for long periods in dry environments has been well documented (CDC 1998; GMA 2009; Podolak and others 2010). Du and others (2010) reported that a 7 log

CFU reduction of *Salmonella* was observed when inoculated almond dust was treated with IPAQuat. They concluded that even in the presence of high levels of organic material, IPAQuat formula was an effective sanitizer, suggesting that alcohol-based sanitizers may be appropriate for in-season hulling and shelling facility sanitation programs (Du and others, 2010).

Table 2.2 Survival of Salmonella *Enteritidis PT 30 Nalr (log CFU/g, n = 6) in almond dusts treated with water or sanitizer and stored at 30ºC (adapted from Du and others 2010)*

Treatment solution [a]	*Salmonella*	
	0 h	48 h
Dust D-4		
Water	4.6 ± 0.3 Aa [b]	6.1 ± 0.5 Ab
Aquat-A [c]	4.6 ± 0.4 Aa	6.0 ± 0.5 Ab
IPAQuat	2.7 ± 1.1 Ba	< 1.3 ± 0.0 Bb
Dust D-6		
Water	5.2 ± 0.2 Aa	7.3 ± 0.5 Ab
Aquat-A	5.2 ± 0.0 Aa	7.5 ± 0.4 Ab
IPAQuat	< 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 *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.

Du and others (2007) also evaluated IPAQuat sanitizer treatments for almond-contact surfaces in hulling and shelling (HS) facilities. Dust collected from HS facilities was inoculated onto new or worn conveyor belting typically used in these facilities. While using the IPAQuat sanitizer reduced contamination by 10 fold (belting surfaces) to 100 fold (steel surfaces) in a laboratory setting, they reported the same treatment did not have the same efficacy and total microbial populations were not significantly reduced in a commercial HS facility. They concluded that the difficulty in uniformly removing dust before applying the IPAQuat formula in

a commercial HS facility may provide explanation for the lack of microbial reduction as compared to the controlled laboratory findings (Du and others 2007).

Summary

Salmonellosis can arise from ingesting very low numbers of *Salmonella* organisms and affects approximately 40,000 individuals annually in the U.S. *Salmonella* outbreaks have been linked to many foods including dry food ingredients and low-moisture products. Control of *Salmonella* is challenging because of its ubiquitous nature. The presence of *Salmonella* in low-moisture products may be attributed to a number of sources including, processing with contaminated (raw) ingredients, not supplying an adequate thermal treatment, or from post-thermal, post-processing contamination. Cross-contamination in the processing environment has been linked to multiple outbreaks of salmonellosis. While inhibition of growth has been reported for a_w values below 0.94, *Salmonella* can persist for many years in low-moisture products.

Control of *Salmonella* in dry-processing environments can be challenging if cleaning methods are limited, not effective in the removal of the microorganisms or if the cleaning methods employed contribute to cross-contamination. Isopropyl alcohol and quaternary ammonium compounds are widely used for their antimicrobial properties. A commercially available formula containing 58.6% isopropyl alcohol and a blend of quaternary ammonium compounds (200 ppm) has been tested with promising results in controlling foodborne pathogens. The sanitizing system evaluated in these studies uses a commercially prepared isopropyl alcohol quaternary ammonium formula and can be sprayed into the environment without adding moisture; therefore, it is expected to have applications for reducing *Salmonella* in dry-processing environments.

Chapter 3 - Use of Isopropyl Alcohol Quaternary Ammonium Formula and Carbon Dioxide Sanitizing Systems for Reducing *Enterococcus faecium* and *Salmonella* Enteritidis on Food Contact Surfaces

Preliminary Research – Phase 1

Objectives

Preliminary experiments focused on the selection of microbiological isolates, as well as investigation into the preparation of inoculums and application of inoculums onto test materials. Test materials such as stainless steel coupons or belting materials representative of those found in dry-processing environments had to be identified and sourced. An appropriate inoculum level and soil had to be determined. The overarching objective was to investigate the efficacy of an isopropyl alcohol quaternary ammonium (IPAQuat) formula and carbon dioxide (CO_2) sanitizing system for reducing *Enterococcus faecium* and *Salmonella* Enteritidis on food contact surfaces.

Materials and Methods

Experimental Design

A culture of *Enterococcus faecium* NRRL B-2354 was inoculated at two different concentrations onto clean or soiled stainless steel coupons or belting materials typically found in dry-processing environments. *Salmonella* Enteritidis ATCC 13076 was inoculated onto soiled stainless steel coupons or belting materials. After inoculums dried onto test materials, coupons were subjected to sanitizing treatments using isopropyl alcohol quaternary ammonium formula (IPAQuat) delivered by using a carbon dioxide (CO_2) sanitizing system. Sanitation consisted of spraying coupons using the IPAQuat-CO_2 system and then allowing the IPAQuat formula to be exposed to inoculated materials for either 1 or 5 min as measured using a timer (timer was set as formula deposited onto coupons). After sanitation, coupons were swabbed, resuspended in 5 ml letheen broth tubes and pour plated using tryptic soy agar pour plates for enumeration of surviving organisms. Control or untreated materials were included to determine pre-treatment populations. The number of surviving organisms was calculated by subtracting post-treatment survivors from pre-treatment populations.

One experiment was conducted, with three samples being evaluated, using two inoculum levels of one organism *(E. faecium)* for each material studied (stainless steel, white, yellow or link belting) and exposure time (1 or 5 min) in experiments involving clean coupons and belting materials. When stainless steel materials were soiled prior to inoculation, one experiment was conducted, with three samples being evaluated, using two inoculum levels for two organisms (*E. faecium* or *S.* Enteritidis) and two exposure times (1 or 5 min) which were independently studied. When belting materials were soiled prior to inoculation, one experiment was conducted, with three samples being evaluated using one inoculum level and one organism (*E. faecium*) and two exposure times (1 or 5 min).

Culture receipt and storage

A culture of *Enterococcus faecium* NRRL B-2354 (ARS Culture Collection, Peoria, IL) was streaked onto Sabouraud dextrose agar (SDA; BBL/Difco, Sparks, MD) and tryptic soy agar plates (TSA; BBL/Difco, Sparks, MD) upon arrival into the laboratory to check for purity. The *Salmonella* Enteritidis ATCC 13076 (American Type Culture Collection, Manassas, VA) isolate was streaked onto Hektoen enteric agar (HEA; BBL/Difco, Sparks, MD) to check for purity. All cultures investigated were analyzed on a Vitek2 System (bioMérieux Corporation, Durham, NC) to confirm correct identification prior to conducting experiments. Cultures were maintained on TSA slants and in tryptic soy broth tubes (TSB; BBL/Difco, Sparks, MD) which were stored in a refrigerator set at 4ºC. A separate culture was maintained frozen by transferring a single colony to TSB containing 15% glycerol (Fisher Chemical, Fisher Scientific, Pittsburgh, PA) and stored at -80ºC.

Inoculum preparation

Using a disposable 10 µl loop (Fisher Scientific, Pittsburgh, PA), an isolated colony of *E. faecium* or *S.* Enteritidis was transferred into separate 10 ml TSB tubes which were then incubated at 35ºC for 22 ± 2 h. After incubation, tubes were vortexed (Fisher Scientific Pulsing Vortex Mixer**,** Fisher Scientific, Pittsburgh, PA) and 0.1 ml of each culture was transferred to a fresh 10 ml tube of TSB per isolate and placed in a 35ºC incubator for an additional 22 ± 2 h.

Following two successive transfers into TSB and subsequent incubation, 1 ml of each vortexed TSB culture tube was transferred to five separate pre-made 15 × 150 mm petri plates (BD, Falcon, Franklin Lakes, NJ) each containing approximately 20 ml of TSA as previously

described by Danyluk and others (2005). This method was chosen to accustom the cells to a reduced water activity environment. Using a separate, sterile disposable L-shaped cell spreader per culture (Fisherbrand, Fisher Scientific, Pittsburgh, PA) (Figure A.1), each culture was spread over the surface of the five TSA plates and incubated 22 ± 2 h at 35ºC.

After incubation, 5 ml of Butterfield's buffer (3M™, St. Paul, MN) was added to each TSA plate (Figure A.2) and the lawn of culture loosened with a sterile cell scraper (BD, Falcon, Franklin Lakes, NJ) (Figure A.3). Loosened cells from all five plates were collected and pooled into one sterile plastic 50 ml conical test tube (BD, Falcon, Franklin Lakes, NJ) (Figure A.4 and A.5) per isolate and refrigerated. Pooled cells from each isolate were vortexed (Figure A.6) and serially diluted using 9 ml or 99 ml Butterfield's buffer (3M™, St. Paul, MN) to achieve 10^6 CFU/ml or 10^8 CFU/ml. Inoculum cell concentration was verified with serial decimal dilutions using Butterfield's buffer (3M), pour plated using TSA (Difco, Becton Dickinson), and then incubated for 48 ± 2 h at 35ºC.

Preparation of stainless steel coupons or conveyor belting materials

Stainless steel coupons, type 304 that were 5 × 5 cm, with no. 4 finish were prepared in the machine shop at Campbell Soup Company, Camden, NJ. Conveyor belting materials were sourced from a dry-processing facility and labeled as yellow, white or link for ease of identification: "yellow" polyurethane (Volta Belting, Pine Brook, NJ), "white" thermoplastic polyurethane (TPU) fabric belt (Habasit America, Suwanee, GA) and "link" style belting materials (Habasit America, Suwanee, GA). Belting materials were cut in the laboratory into 5 × 15 cm strips with lines drawn demarking every 5 cm, so that the belting was divided into three 5 × 5 cm square sections (Figure A.7).

Stainless steel coupons and belting materials were first cleaned with Citranox® liquid-acid detergent (Fisher Scientific, Pittsburgh, PA). Then, stainless steel coupons were prepared by soaking overnight for 16-18 h in 1N NaOH (Fisher Scientific, Pittsburgh, PA). After soaking, stainless steel coupons were rinsed with tap water for approximately 10 min until the rinse water reached a neutral pH (7.5) as determined using a calibrated pH meter (Metrohm Ion 827 pH Lab Meter, Riverview, FL). Once a neutral pH was established, coupons were rinsed twice with deionized water. Cleaned coupons were placed on a foil (Fisherbrand, Fisher Scientific, Pittsburgh, PA) lined metal tray to dry. Once dried, coupons were marked in the upper left corner with a black (10^6 CFU/ml) or red (10^8 CFU/ml) dot to indicate level of inoculum. Trays

of coupons were covered with foil and autoclaved at 121°C for 15 min. Trays of coupons were left covered on the lab bench overnight 16-18 h to thoroughly dry before inoculating.

Strips of belting were submersed in boiling distilled water for 20 min, collected using sterile tongs and placed on sterile foil lined trays containing a non-sterile absorbent blotter (Fisher Scientific, Pittsburgh, PA). After air drying for 60 min, sterile forceps were used to move strips of belting materials to sterile foil lined trays. Trays of belting materials were left covered on the lab bench overnight 16-18 h to thoroughly dry before inoculating.

Preparation of soiled surfaces

Stainless steel coupons and belting materials were sanitized prior to soiling using the procedures described previously. Sanitized surfaces were artificially soiled by spraying the top surfaces with a flour and water solution till visibly wet. The solution was first prepared by adding 8 g of bread flour (King Arthur's, Norwich, VT) to a clean flask and adding 92 ml of distilled water. After a through mixing of the solution, the flask was autoclaved and the liquid fraction which remained on the top of the flask after autoclaving transferred to a sanitized 8-ounce spray bottle (Fisherbrand, Fisher Scientific, Pittsburgh, PA). Soiled test materials, which were contained on foil-lined trays, were air-dried on the bench top (24 ± 2° C) for 2 h and then spot-inoculated with 10 µl of the prepared inoculums.

Inoculation of materials

Serial dilutions were prepared from the refrigerated inoculums' of *E. faecium* or *S.* Enteritidis using Butterfield's buffer (3M, St. Paul, MN). Coupons and belting materials were inoculated in triplicate for each inoculum concentration (low: 10^6 CFU/ ml and high: 10^8 CFU/ ml) and exposure time (1 and 5 min), using 10 µl of *E. faecium* or *S.* Enteritidis prepared from pooled cells. Using a repeat pipettor (Rainin edp2, Mettler Toledo, USA), 10 µl of inoculum was deposited to the center of the 5 × 5 cm square for each test material (Figure A.8 and A.9). Once materials were inoculated, trays containing materials were loosely covered with sterilized aluminum foil and dried on the lab bench top overnight 16-18 h (24 ± 2°C).

Treatments using IPAQuat-CO_2 sanitizing system

Fresh bottles of IPAQuat formula (D2, Best Sanitizers Inc., Penn Valley, CA and 1 Priority Biocidal LLC, Fort Worth, TX) were opened and attached to the CO_2 sanitizing system

(Figure A.10 and A.11). The valve of the sanitizing system was fully opened and the regulator knob set at 30 psi. Trays containing coupons and belting materials were placed on a laboratory cart and sprayed from 0.91 m, starting on the left and working towards the right. The timer was started as the IPAQuat formula was deposited onto the surfaces. Treatments consisted of spraying test materials till visibly wet using IPAQuat-CO_2 sanitizing system, exposing inoculated test materials to the IPAQuat formula for 1 or 5 min. The amount of formula deposited on the coupons ranged from 0.05 ± 0.01 g for a 1 min exposure and 0.07 ± 0.01 g for a 5 min exposure.

Enumeration of surviving organisms

After sanitation, the center of coupons and belting materials were immediately swabbed using Dacron® polyester tipped swabs and resuspended in 5 ml letheen broth tubes (3M™, St. Paul, MN) to neutralize the sanitizer. Before swabbing, the swab tip was pressed and twisted against the side wall of the tube to wring out excess neutralizing buffer. After swabbing the surface for 15 s as measured using a second timer, the swab was returned to the letheen broth tube. Swabs were vortexed for 20 s prior to plating to release bacteria from the swab tip. Serial ten-fold dilutions were prepared and plated using TSA pour plates (Figure A.12 and A.13) to enumerate surviving organisms. Plates were incubated for 48 ± 2 h at 35°C and then counted.

Counting plates and calculations

Colonies were counted by hand using a Reichert Darkfield Quebec Colony Counter (Fisher Scientific, Pittsburgh, PA). The number of surviving microorganisms was calculated by subtracting post-treatment survivors from pre-treatment populations.

Statistical analyses

Data were analyzed using analysis of variance methods in the PROC MIXED procedures of SAS® statistical analysis software (SAS® version 9.2, SAS Institute, Cary, N.C.). To analyze the data, a value of 0.7 was assigned for all counts that were below the detection limit of <0.7 log CFU/25 cm^2. Fixed effects were exposure time and material; random effects were the number of number of samples analyzed for the one experiment. Differences between mean values were considered significant at α-value of 0.05.

Results

The initial mean population (0 min) of *Enterococcus faecium* on stainless steel coupons was 4.09 log CFU/25 cm^2 when coupons were clean versus 4.69 log CFU/25 cm^2 when coupons were soiled prior to inoculation. Populations after treatments with IPAQuat-CO_2 sanitizing system decreased ($p<0.05$) for all stainless steel materials whether they were clean or soiled prior to inoculation (Figure 3.1). Post-treatment populations from stainless materials that were clean prior to inoculation were below the level of detection (0.70 log CFU/25 cm^2) for 1 or 5 min treatments. Post-treatment populations from soiled materials on stainless steel had low levels of survivors, 0.87 log CFU/25 cm^2 and 1.13 log CFU/25 cm^2, after 1 or 5 min respectively. Results indicate that time of sanitizer exposure used in this study did not affect ($p>0.05$) inactivation of *E. faecium* on stainless steel (Figure 3.1).

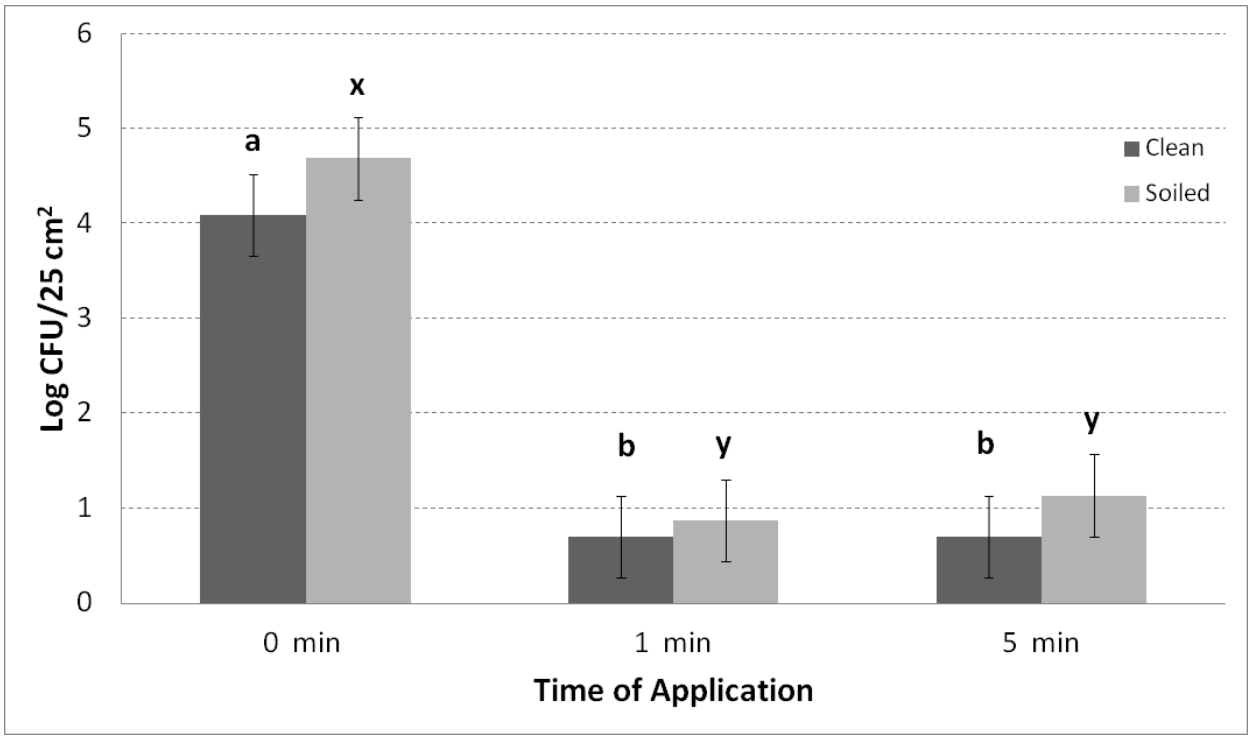

Figure 3.1 *Mean populations of* Enterococcus faecium NRRL B-2354 *(log CFU/25 cm^2) on stainless steel after treatments with IPAQuat-CO_2 sanitizing system. Limit of detection 0.70.* $^{a\,b}$*Superscripts indicate differences ($p<0.05$) for clean material treatments.* $^{x\,y}$ *Superscripts indicate differences ($p<0.05$) for soiled material treatments. The values plotted are means ± standard error with n=3.*

The initial mean population (0 min) of *Enterococcus faecium* on white belting material was 3.14 log CFU/25 cm^2 for clean coupons versus 7.74 log CFU/25 cm^2 for coupons soiled prior to inoculation. Populations after treatments with IPAQuat-CO_2 sanitizing system decreased ($p<0.05$) for all white belting materials whether they were clean or soiled prior to inoculation (Figure 3.2). Post-treatment populations from white belting materials that were clean prior to inoculation were below the level of detection (0.70 log CFU/25 cm^2) for 1 or 5 min treatments. Post-treatment populations from soiled materials had low levels of survivors, 2.95 log CFU/25 cm^2 and 1.35 log CFU/25 cm^2 after 1 or 5 min respectively. Results indicate that time of sanitizer exposure used in this study did not affect ($p>0.05$) inactivation of *E. faecium* on clean white belting. However, time of sanitizer exposure was significant ($p<0.05$) in reducing populations of *E. faecium* when white belting materials were soiled prior to inoculation (Figure 3.2). Results show that 5 min was more effective than 1 min.

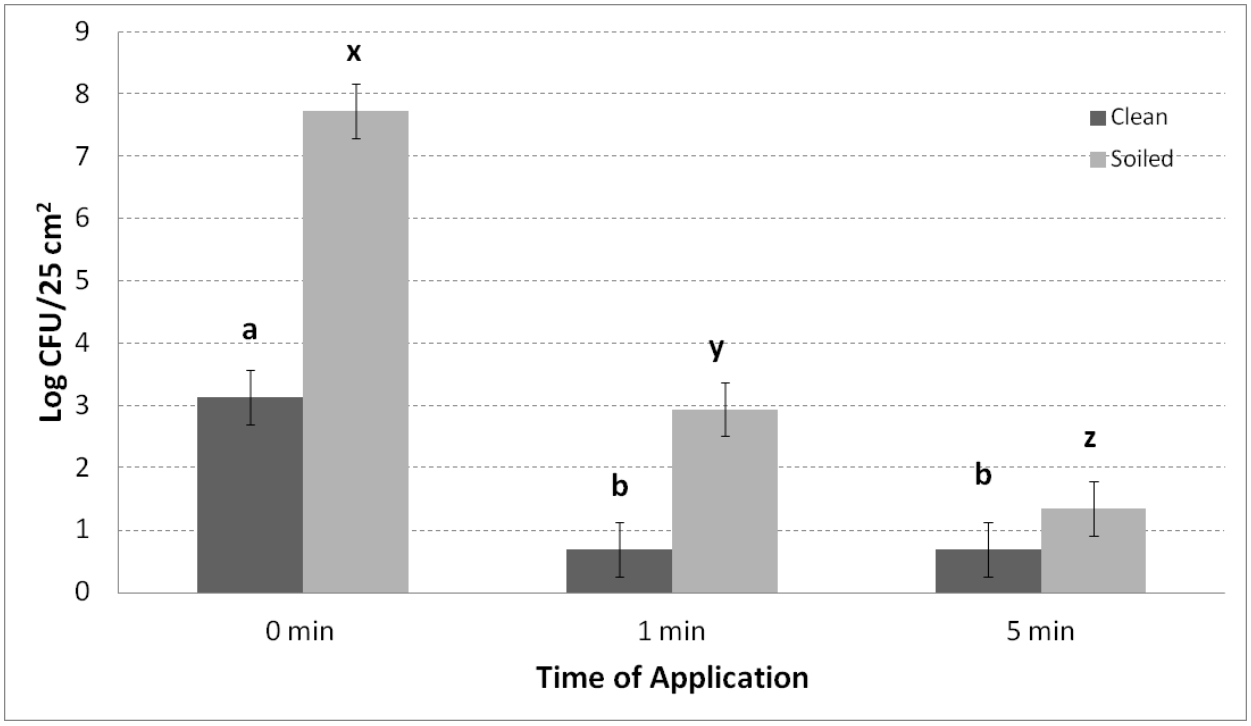

Figure 3.2 *Mean populations of* Enterococcus faecium *NRRL B-2354 (log CFU/25 cm^2) on white belting after treatments with IPAQuat-CO2 sanitizing system. Limit of detection 0.70.* $^{a\,b}$ *Superscripts indicate differences ($p<0.05$) for clean material treatments.* $^{x\,y\,z}$ *Superscripts indicate differences ($p<0.05$) for soiled material treatments. The values plotted are means ± standard error with n=3.*

The initial mean population (0 min) of *Enterococcus faecium* on yellow belting material was 1.49 log CFU/25 cm^2 when coupons were clean versus 7.71 log CFU/25 cm^2 for coupons soiled prior to inoculation. Populations after treatments with IPAQuat-CO_2 sanitizing system did not decrease ($p>0.05$) for clean belting but did decrease ($p<0.05$) after treatment for soiled yellow belting materials (Figure 3.3). Post-treatment populations from yellow materials that were clean prior to inoculation were below the level of detection (0.70 log CFU/25 cm^2) for 1 or 5 min treatments. Post-treatment populations from soiled materials had low levels of survivors, 2.34 log CFU/25 cm^2 and 1.93 log CFU/25 cm^2 after sanitation exposure of 1 or 5 min, respectively. Results indicate that time of sanitizer exposure used in this study did not affect ($p>0.05$) inactivation of *E. faecium* on soiled yellow belting (Figure 3.3).

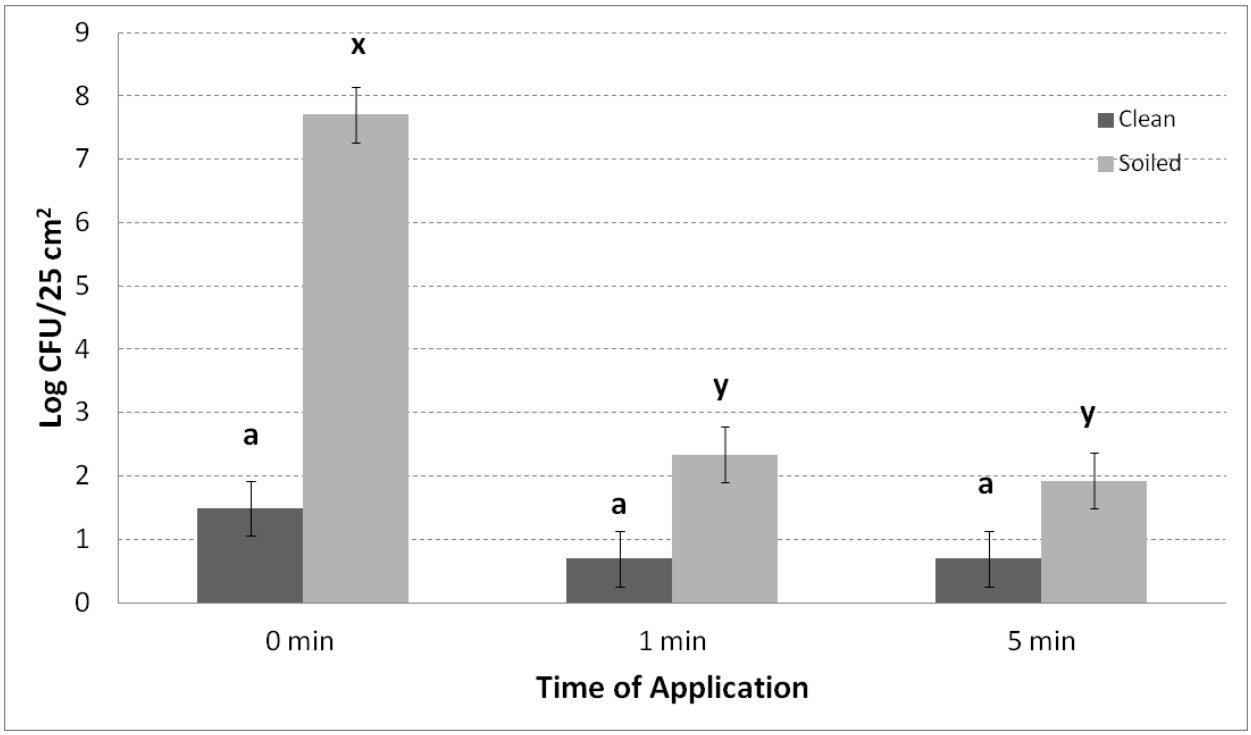

Figure 3.3 *Mean populations of* Enterococcus faecium *NRRL B-2354 (log CFU/25 cm^2) on yellow belting after treatments with IPAQuat-CO_2 sanitizing system. Limit of detection 0.70.* $^{a\ b}$*Superscripts indicate differences ($p<0.05$) for clean material treatments.* $^{x\ y}$ *Superscripts indicate differences ($p<0.05$) for soiled material treatments. The values plotted are means ± standard error with n=3.*

The initial mean population (0 min) of *Enterococcus faecium* on link material was 3.95 log CFU/25 cm^2 when materials were clean versus 7.83 log CFU/25 cm^2 for coupons soiled prior to inoculation. Populations after treatments with IPAQuat-CO_2 sanitizing system decreased ($p<0.05$) for link materials whether they were clean or soiled prior to inoculation (Figure 3.4). Post-treatment populations from link materials that were clean prior to inoculation were 0.75 log CFU/25 cm^2 after 1 min but below the level of detection (0.70 log CFU/25 cm^2) after 5 min treatments. Post-treatment populations from soiled materials had survivors, 3.43 log CFU/25 cm^2 and 4.47 log CFU/25 cm^2, after 1 or 5 min respectively. Results indicate that time of sanitizer exposure used in this study did not affect ($p<0.05$) inactivation of *E. faecium* on link materials (Figure 3.4).

Results from this study indicate that material and time did not have a significant ($p>0.05$) interaction. Mean populations of *Salmonella* Enteritidis over all times (0, 1 and 5 min) for soiled materials were 6.56 log CFU/25 cm^2 for link belting, 3.97 log CFU/25 cm^2 for stainless steel coupons, 4.87 log CFU/25 cm^2 for white belting, and 5.48 log CFU/25 cm^2 for yellow belting (Figure 3.5). While mean populations differed ($p<0.05$) between stainless and link materials, the difference in populations for stainless, white and yellow, or yellow, white and link was not different ($p>0.05$) (Figure 3.5).

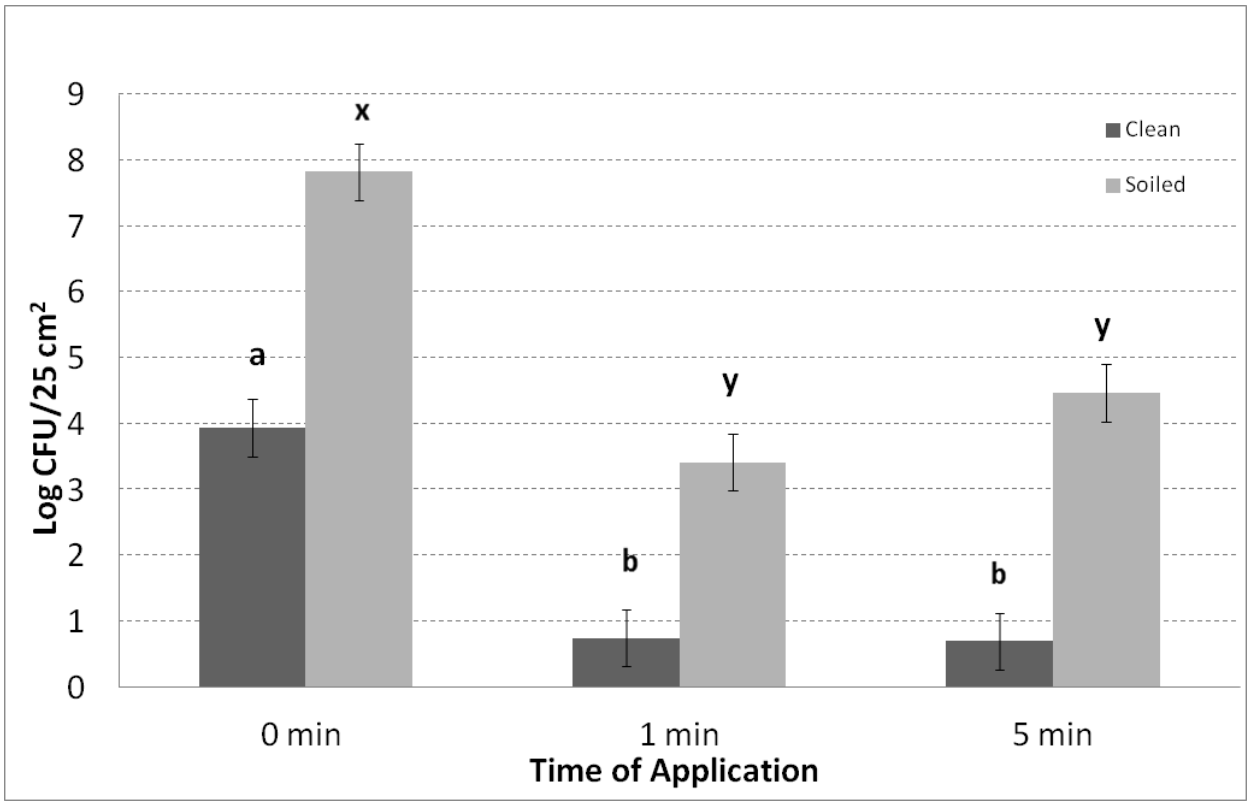

Figure 3.4 *Mean populations of* Enterococcus faecium *NRRL B-2354 (log CFU/25 cm^2) on link belting after treatments with IPAQuat-CO_2 sanitizing system. Limit of detection 0.70.* $^{a\ b}$*Superscripts indicate differences ($p<0.05$) for clean material treatments.* $^{x\ y}$ *Superscripts indicate differences ($p<0.05$) for soiled material treatments. The values plotted are means ± standard error with n=3.*

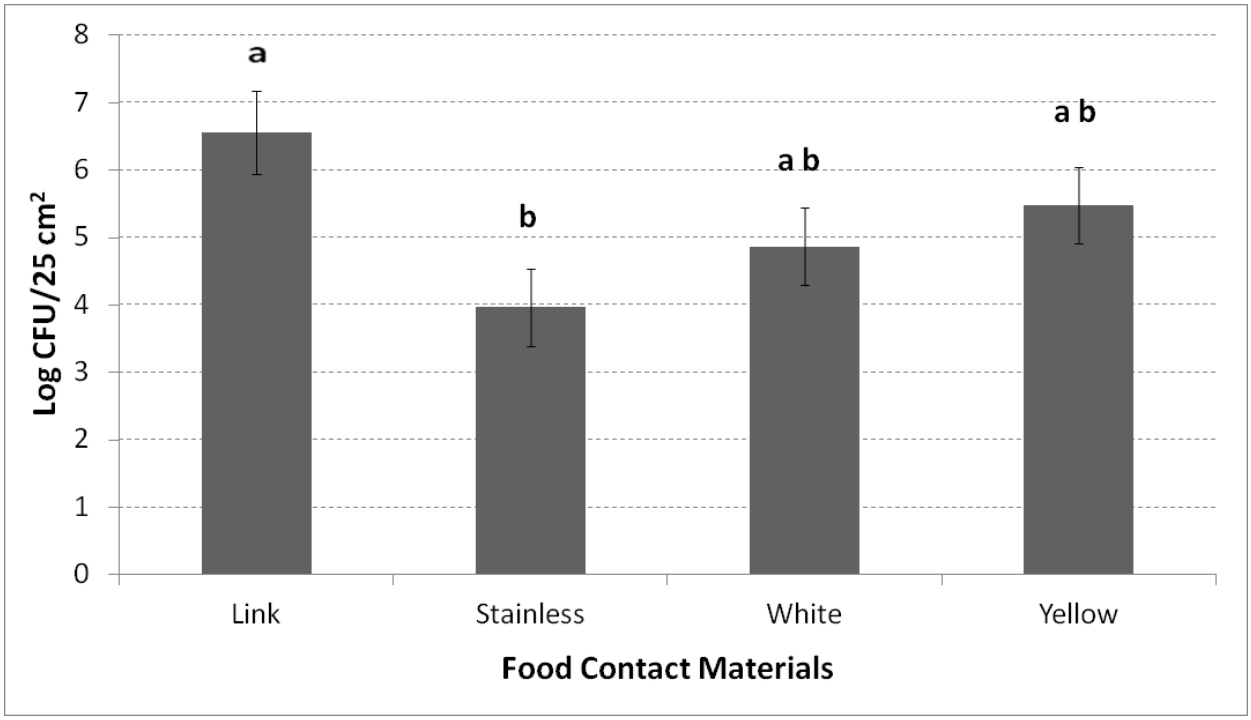

Figure 3.5 *Mean populations of* Salmonella *Enteritidis (log CFU/25 cm^2) on all materials prior to treatments with IPAQuat-CO_2 sanitizing system.* $^{a\ b}$ *superscripts indicate differences ($p<0.05$) for initial populations. The values plotted are means ± standard error with n=3.*

The initial population (0 min) of *Salmonella* Enteritidis from all soiled materials averaged 7.75 log CFU/25 cm^2. Post-treatment survivors from all soiled materials were 4.61 log CFU/25 cm^2 for a 1 min treatment and 3.30 log CFU/25 cm^2 for a 5 min treatment (Figure 3.6). Results indicate that time of sanitizer exposure (1 or 5 min) used in this study did not affect ($p>0.05$) inactivation of *Salmonella* on coupons (Figure 3.6).

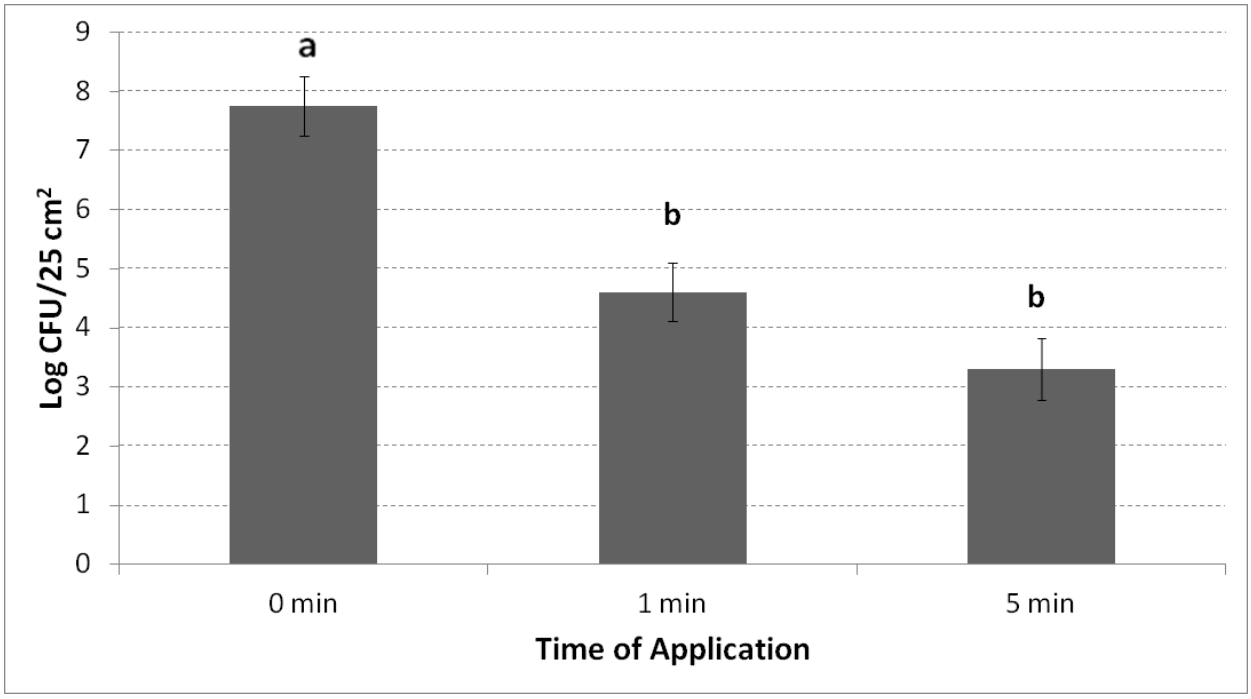

Figure 3.6 *Mean populations of* Salmonella *Enteritidis (log CFU/25 cm^2) on all materials (stainless steel and white, yellow, and link belting material) post-treatments with IPAQuat-CO_2 sanitizing system.* $^{a\ b}$ *Superscripts indicate differences ($p<0.05$) for initial populations. The values plotted are means ± standard error with n=3.*

Discussion

It was expected that treatments with IPAQuat-CO_2 sanitizing system would be effective in reducing populations of microorganisms on stainless steel coupons and belting materials. The IPAQuat formula contains 58.6% isopropyl alcohol and a blend of quaternary ammonium compounds (200 ppm); both widely used for their antimicrobial properties. With the exception of clean yellow belting which had initial low inoculum level (0.75 log CFU/25 cm^2), sanitizer treatments with an exposure time of 1 or 5 min were significant ($p<0.05$) in reducing bacterial populations from the pre-treatment samples for materials tested whether they were clean or soiled prior to inoculation.

With the exception of soiled white belting material, time of sanitizer exposure (1 min or 5 min) did not affect ($p>0.05$) post-treatment populations. When materials were cleaned prior to inoculation, post-treatment populations were below the level of detection (<0.7 log CFU/25 cm^2) with the exception of the link belting which had low levels of survivors (0.75 log CFU/25 cm^2) with a 1 min treatment. Low levels of survivors from link belting could be attributed to the

uneven surfaces and crevices characteristic of the link belting. The log reduction observed for clean materials ranged from 0.79 log CFU/25 cm^2 to 3.39 log CFU/25 cm^2.

Soiled surfaces (all materials) had low levels of surviving organisms (*Enterococcus faecium* and *Salmonella* Enteritidis) for 1 and 5 min sanitizer treatments. The log reduction observed for all soiled materials and both organisms ranged from 3.36 log CFU/25 cm^2 to 6.39 log CFU/25 cm^2.

Conclusion

The IPAQuat-CO_2 sanitizing system was effective in reducing *Enterococcus faecium* and *Salmonella* Enteritidis from stainless steel coupons and belting materials when the surfaces were exposed to the IPAQuat sanitizer for 1 or 5 min. When surfaces were clean prior to inoculation, IPAQuat-CO_2 sanitizing system was effective in reducing populations below the limit of detection (<0.70). In contrast, low levels of surviving organisms from surfaces that were soiled prior to inoculation suggest that surfaces must be adequately cleaned prior to the application of a sanitizer.

Next Steps

For additional experiments, the IPAQuat-CO_2 sanitizing system will be evaluated for efficacy in reducing 5 log CFU/25 cm^2 of *Salmonella* using sanitation exposure times of 30 s, 1 and 5 min. The experimental design will focus on inoculating two materials (stainless steel coupons and yellow belting materials), two conditions (clean and soiled), with *Salmonella* isolated from clinical, product, and environmental sources. A soil matrix typical of a dry-processing environment will be identified and sourced.

Chapter 4 - Efficacy of an Isopropyl Alcohol Quaternary Ammonium Formula and Carbon Dioxide Sanitizer System for Reducing *Salmonella* on Food Contact Surfaces

Abstract

Cleaning and sanitation operations directly impact the safety of manufactured foods. Dry-processing environments, such as those that process low-moisture products, are particularly challenging to clean and sanitize because water introduced into systems not designed for wet cleaning can favor growth and establishment of pathogenic microorganisms such as *Salmonella*. Inadequate sanitation in dry-processing environments has been linked to several multistate outbreaks of salmonellosis resulting from cross-contamination. The objective of this study was to evaluate the efficacy of an isopropyl alcohol quaternary ammonium (IPAQuat) formula and carbon dioxide (CO_2) sanitizer system for eliminating potential *Salmonella* contamination on food contact surfaces. Coupons of stainless steel and conveyor belting material typically used in dry-processing environments were spot-inoculated in the center of 5 × 5 cm coupons with approximately 10 log CFU/ml of a six-serotype composite of *Salmonella* and treated using an IPAQuat-CO_2 sanitizer system. After treatments, using sanitizer exposure times of 30 s, 1 or 5 min, coupons were swabbed and swabs were enumerated for surviving organisms using tryptic soy agar pour plates and Hektoen enteric agar for *Salmonella* confirmation. Duplicate inoculated surfaces were soiled (after the inoculums dried) with a breadcrumb flour blend and allowed to sit on the lab bench for 16 to 18 h before sanitation. While pre-treatment mean *Salmonella* populations were 7.0 log CFU/25 cm^2, post-treatment mean *Salmonella* populations were 0.83, <0.7, and <0.7 log CFU/25 cm^2 after 30 s, 1 or 5 min sanitation treatments, respectively, for all surfaces. While sanitation treatments of 30 s using IPAQuat-CO_2 sanitizer system resulted in low levels of survivors, approximately 6.0 log CFU/25 cm^2 reductions were observed for 1 and 5 min sanitation treatments. Therefore, IPAQuat-CO_2 sanitizer system was effective in reducing 6 logs of *Salmonella* from inoculated surfaces with sanitation treatments of 1 min or more. Because water is not introduced into the processing environment, the IPAQuat-CO_2 sanitizer system could have sanitation applications in dry-processing environments or facilities that process low-moisture products.

Introduction

During the last decade, a number of outbreaks of salmonellosis impacting large numbers of individuals, and resulting in several deaths have been associated with low-moisture ready-to-eat products such as powdered infant formula, dried milk products, raw almonds, cereals, dry seasonings, dry pet food products, and pet treats (CDC 2007 and 2008; Cahill and others 2008; GMA 2009; Barton and others 2010; Podolak and others 2010). Although *Salmonella* cannot grow in dry goods (water activity less than 0.94), *Salmonella* is resistant to desiccation and able to survive for a long time on stainless steel or under dry conditions (Iibuchi and others 2010; Kusumaningrum and others 2003; Podolak and others 2010). The survival and transfer of *Salmonella* and other bacteria from contaminated surfaces to new foods has been widely studied and becomes a significant food safety risk when considering the potential for products to become cross-contaminated from the processing equipment. Investigations into the epidemiological and environmental patterns associated with outbreaks of salmonellosis have suggested that cross-contamination and inadequate sanitation have played major roles in the contamination of products with *Salmonella* (Kusumaningrum and others 2003; Podolak and others 2010).

Many food processing facilities utilize detergents and sanitizers that are mixed with water to clean equipment. However, wet cleaning procedures in facilities that process dry ingredients or low-moisture products is not always appropriate; excess humidity and moisture without adequate drying alter a food plant environment. Cleaning and sanitation in dry-processing environments becomes particularly challenging because water introduced into systems not designed for wet cleaning can favor growth and establishment of pathogenic microorganisms such as *Salmonella* (GMA 2009). Microbial niche environments can develop in cracks, crevices, pits, holes, and junctions that have accumulated food, dust, debris, and water. These areas may be hard to inspect, clean or sanitize, and can therefore protect microorganisms from being destroyed (Pouch Downes and Ito 2001; GMA 2009; Umland 2003).

The cleaning methods and tools used to “dry clean” equipment and environments are typically limited to sweeping, scraping, vacuuming, and wiping with cloths. Compressed air, brushing, and blasting with carbon dioxide (CO_2), sand or bicarbonate soda are also dry cleaning methods. Blasting technology, however, requires a secondary clean-up, and wiping with cloths has limited application and can only be useful for small areas. Problems associated with sweeping and scraping are aerosolized dust and debris which may lead to cross-contamination.

It has been shown that compressed air, if strong enough, can send a small clog to parallel processing lines and be a potential source of cross-contamination (Jackson and others 2007; Roder and others 2010).

A sanitizing system which utilizes CO_2 and an isopropyl alcohol quaternary ammonium (IPAQuat) formula is commercially available (1 Priority Biocidal, Fort Worth, TX or Biomist, Inc., Wheeling, IL) and could have potential for reducing *Salmonella* on food contact surfaces. The sanitizing system's technology which uses CO_2 as a propellant or carrier to deliver a spray of IPAQuat formula makes it possible to sanitize without adding water to processing environments. The active IPAQuat formula contains 58.6% isopropyl alcohol and a low concentration (200 ppm) of quaternary ammonium compound (D2; 1 Priority Biocidal, Fort Worth,TX; Best Sanitizers, Penn Valley, CA), both known to be effective sanitizing agents. After the alcohol evaporates, the quaternary ammonium compound leaves an antimicrobial film and residual sanitizing effect. If this technology and tested system prove to be effective, it could have sanitation application in dry-processing or low-moisture environments. Because water is not introduced into the environment, risk of spreading pathogenic bacteria is minimized.

The IPAQuat-CO_2 system has been shown to be effective against several pathogens. Experiments conducted using almond dust inoculated with *Salmonella* resulted in reports of 7 log CFU reductions when inoculated dust was treated with IPAQuat formula (Du and others 2010). An independent third party laboratory tested and found the active formula to be effective against *Escherichia coli* and *Staphylococcus aureus* with a 6.9 log CFU/carrier reduction after 1 min sanitation treatment (Bioscience 2000). The objective of this study was to evaluate the efficacy of IPAQuat-CO_2 sanitizing systems to eliminate potential *Salmonella* contamination from food contact surfaces for application in facilities that process dry ingredients or low-moisture products.

Materials and Methods

Experimental Design

Stainless steel coupons and belting materials were inoculated with *Salmonella* and not treated (0 s, control) or treated with a dry sweep and/or treatments with a sanitation system using sanitizer exposures of 30 s, 1 or 5 min. For each experiment, at least 3 samples were treated and

analyzed and each experiment was conducted three times for a total of three replications with n=9 or more.

Culture receipt and storage

Cultures of *Salmonella* serotypes Hartford FSL R8-5223 (peanut isolate, ILSI funded project), Tennessee FSL R8-5221 (peanut isolate, International Life Sciences Institute (ILSI) funded project), Enteritidis FSL S5-415 (human isolate), Agona FSL S5-517 (human isolate), Newport FSL R8-4035 (isolated from bovine farm environment) and FSL R8-4086 Enteritidis (isolated from avian farm environment) were obtained from Cornell Food Safety Lab, Ithaca, NY (collection, storage, and distribution of isolates obtained from the Cornell Food Safety Laboratory is supported through USDA Food Safety Special Research Grants). Cultures were streaked onto Hektoen enteric agar (HEA; BBL/Difco, Sparks, MD) to check for purity. Representative colonies from the HEA purity plates were analyzed on a Vitek2 System (bioMérieux Corporation, USA) to confirm *Salmonella* identification prior to conducting experiments. Cultures were maintained on tryptic soy agar (TSA; BBL/Difco, Sparks, MD) slants and in 10 ml of tryptic soy broth (TSB; BBL/Difco, Sparks, MD) which were stored in a refrigerator set at 4ºC. A separate culture was maintained frozen by transferring a single colony to a tube of TSB supplemented with 15% glycerol (Fisher Chemical, Fisher Scientific, Pittsburgh, PA) and stored at -80ºC.

Inoculum preparation

From the purity plates, a 10 µl loop (Fisher Scientific, Pittsburgh, PA) was used to transfer an isolated colony of *Salmonella* cultures into separate 10 ml TSB tubes which were then incubated at 35ºC for 22 ± 2 h. After incubation, tubes were vortexed (Fisher Scientific Pulsing Vortex Mixer**,** Fisher Scientific, Pittsburgh, PA) and 0.1 ml of each culture was transferred to a fresh 10 ml tube of TSB per isolate and placed in a 35ºC incubator for an additional 22 ± 2 h. Following two successive transfers into TSB, cultures were stored in a refrigerator set at 4ºC. Each time an experiment was planned, 0.1 ml of each refrigerated TSB culture was transferred to a fresh 10 ml tube of TSB per isolate and placed in a 35ºC incubator for 22 ± 2 h. After incubation, 1 ml of each vortexed TSB culture tube was transferred to one pre-made 15 × 150 mm petri plate (BD, Falcon, Franklin Lakes, NJ) containing approximately

20 ml of TSA as previously described by Danyluk and others (2005). This method was chosen to accustom the cells to a reduced water activity environment.

Using a separate, sterile disposable L-shaped cell spreader per culture (Fisherbrand, Fisher Scientific, Pittsburgh, PA), each culture was spread over the surface of a TSA plate and incubated 22 ± 2 h at 35ºC. After incubation, 5 ml of Butterfield's buffer (3M™, St. Paul, MN) was added to each TSA plate and the lawn of culture loosened with a sterile disposable L-shaped cell spreader (BD, Falcon, Franklin Lakes, NJ). Loosened cells from all isolates were collected and pooled into one sterile plastic 50 ml conical test tube (BD, Falcon, Franklin Lakes, NJ).

Preparation of test surfaces

Stainless steel coupons (type 304; 5 × 5 cm) with no. 4 finish were prepared by the machine shop at Campbell Soup Company, Camden, NJ. Conveyor belting materials ("yellow" polyurethane, Volta Belting, Pine Brook, NJ) were sourced from a dry-processing facility. Conveyor belting materials were cut in the laboratory into 5 ×15 cm with lines demarking every 5 cm (Figure A.14). Stainless steel coupons and belting materials were first cleaned with Citranox® liquid-acid detergent (Fisher Scientific, Pittsburgh, PA), and then rinsed with flowing tap water for approximately 10 min followed by two rinses with deionized water. Cleaned coupons and belting materials were placed on aluminum trays lined with absorbent blotter (Fisher Scientific, Pittsburgh, PA) to dry (approximately 1 h). After drying, stainless steel coupons were moved to aluminum trays that were lined with aluminum foil (Fisher Scientific, Pittsburgh, PA). A second foil sheet was used to cover the tray before autoclaving at 121°C for 15 min. Autoclaved coupons were left on the lab bench for 1 h to cool. Belting materials were wiped till thoroughly wet using 70% alcohol prep swabs (BD, Fisher Scientific, Pittsburgh, PA) and rinsed using a Kimwipe® (Fisher Scientific, Pittsburgh, PA) wetted with sterile distilled water. Sanitized belting materials were left on the lab bench to dry (approximately 1 h).

Inoculation of materials

Pooled cells were diluted ten-fold using 9 ml Butterfield's buffer (3M™, St. Paul, MN) to achieve approximately 10^{10} CFU/ml. Coupons and belting materials were inoculated in triplicate for each treatment time (30 s, 1 and 5 min), using 10 µl of the serially diluted pooled cells distributed to the center of each test surface using a repeat pipettor and tips (Rainin edp2, Mettler Toledo, USA). Inoculated materials were loosely covered with sterile foil sheets and air-

dried on the lab bench top overnight for 16-18 h (24 ± 2°C). Inoculums' cell concentration was verified with serial decimal dilutions using Butterfield's buffer (3M) and pour plated using TSA (Difco, Becton Dickinson). Plates were incubated for 48 ± 2 h at 35ºC and then counted.

Preparation of soiled surfaces

After inoculums dried onto coupons and belting material (2 h, 24 ± 2ºC) (Figure A.16), surfaces were soiled by depositing 1 g of a breadcrumb (Great Value Brand, Walmart, Bentonville, AK) and bread flour (King Arthur's, Norwich, VT) blend (Figures A.17-18) directly on top of the dried inoculums. The blend was first prepared by mixing 80 g of bread crumbs with 20 g of bread flour. The bread crumb and flour blend was determined to be *Salmonella* free using a BAX® detection system (bioMérieux Corporation, USA).

Treatments using IPAQuat-CO2 sanitizing system

The breadcrumb flour blend (soil) was initially removed before sanitation treatments by turning the test surfaces sideways and tapping once. Treatments consisted of a dry sweep using a small hand-held broom (Procter & Gamble, Cincinnati, OH) and/ or an application of IPAQuat formula to inoculated surfaces using IPAQuat-CO_2 sanitizing system (Figure A.19). To use the IPAQuat-CO_2 sanitizer system, the valve of system was fully opened and the regulator knob set at 30 psi. Test materials were placed on sterile foil lined trays on the top shelf of a lab cart and sprayed from 0.91 m away, starting on the left and working towards the right. The timer was started as the IPAQuat formula deposited onto the test surfaces. Sanitation treatments consisted of exposing inoculated materials to the IPAQuat formula delivered using the CO_2 sanitizer system for 30 s, 1 or 5 min. The amount of formula deposited on the coupons ranged from 0.03 ± 0.01 g for a 30 s exposure 0.05 ± 0.01 g for a 1 min exposure and 0.07 ± 0.01 g for a 5 min exposure.

Enumeration of surviving organisms

After sanitation, the center of the coupons and belting materials were immediately swabbed using Dacron® polyester tipped swabs and resuspended in 5 ml letheen broth tubes (3M™, St. Paul, MN) to neutralize the sanitizer. Before swabbing, swab tips were pressed and twisted against the side wall of the tube to wring out excess neutralizing buffer. After swabbing the surface for 5 s (30 s treatments) or 15 s (1 or 5 min treatments) as determined using a second

timer, swabs were returned to letheen broth tubes. Swabs were vortexed for 20 s prior to plating to release bacteria from the swab tip. Serial ten-fold dilutions were prepared and plated using TSA pour plates (Figure A.12 and A.13) to enumerate surviving organisms. Plates were counted after 48 ± 2 h at 35ºC.

Counting plates and calculations

Colonies were counted by hand using a Reichert Darkfield Quebec Colony Counter (Fisher Scientific, Pittsburgh, PA). The number of surviving *Salmonella* was calculated by subtracting post-treatment survivors from pre-treatment populations. Colonies were confirmed as *Salmonella* using HEA.

Statistical analyses

Each experiment was repeated at least three times on different days and no less than three coupons were used in each experiment. Microbial counts were transformed to logarithms before means and standard deviations were computed. Counts were reported as log CFU/25 cm^2.

Data were analyzed using analysis of variance methods in the PROC MIXED procedures of SAS® statistical analysis software (SAS® version 9.2, SAS Institute, Cary, N.C.). To analyze the data, a value of 0.7 was assigned for all counts that were below the detection limit of <0.7 log CFU/25 cm^2. Fixed effects were exposure time and material; random effects were the number of replications and number of samples. Differences between mean values were considered significant at α-value of 0.05.

Results

Mean populations of *Salmonella* attached to all stainless steel coupons and belting material (clean and soiled) before treatments using the IPAQuat-CO_2 system were 6.84 log CFU/25 cm^2. A 30 s treatment using IPAQuat-CO_2 system resulted in 6 log reductions of *Salmonella* and the mean populations were 0.83 ± 0.1 CFU/25 cm^2. After 1 or 5 min treatments, mean *Salmonella* population was below the level of detection (<0.70). Sanitation treatments using IPAQuat-CO_2 system reduced ($p<0.05$) *Salmonella* populations from all materials (clean or soiled) after sanitizer exposures of 30 s, 1 or 5 min (Figure 4.1).

Mean *Salmonella* populations attached to clean stainless steel coupons and belting material prior to sanitation treatments ranged from 6.93 to 6.99 ± 0.1 log CFU/25 cm^2 (Table

4.1). Pre-treatment mean *Salmonella* populations attached to soiled materials were lower ($p<0.05$) and ranged from 6.71 to 6.72 ± 0.1 log CFU/25 cm^2 (Table 4.1). Sanitation treatments applied to inoculated clean stainless steel materials resulted in a 6.18 ± 0.2 log CFU/25 cm^2 reduction after a 30 s sanitizer exposure. After 1 or 5 min sanitation treatments, mean populations were below the level of detection (0.70) representing a 6.23 ± 0.2 log CFU/25 cm^2 log reduction.

Sanitation treatments applied to soiled stainless steel samples resulted in 6.02 ± 0.2 log CFU/25 cm^2 reductions after sanitizer exposures of 30 s, 1 or 5 min. The mean *Salmonella* populations were below the level of detection (0.70) with a 30 s treatment using IPAQuat-CO_2 system. Sanitation treatments applied to soiled belting materials yielded similar results as those observed for soiled stainless steel materials. Mean populations of *Salmonella* were below the level of detection (0.70) with a 30 s treatment and resulted in 6.01 ± 0.2 log CFU/25 cm^2 reductions. Overall, time of sanitizer exposure did not affect ($p>0.05$) inactivation of *Salmonella* attached to coupons for most of the materials studied (Table 4.1).

Low levels of survivors (1.31 ± 0.2 log CFU/25 cm^2) enumerated from clean belting material after a 30 s sanitation treatment were higher ($p<0.05$) than all other materials and conditions (clean or soiled) studied. It can be said that a 30 s treatment using IPAQuat-CO_2 sanitizing system resulted in a 5.68 log reduction under the conditions of this study (Table 4.1). Treatments of 1 or 5 min using IPAQuat-CO_2 sanitizing system resulted in 6.0 log reductions (Table 4.1). Dry-sweeping coupons resulted in mean *Salmonella* populations of 6.81 log CFU/25 cm^2 and did not substantially (≤0.03 log CFU/25 cm^2) reduce *Salmonella* spot-inoculated on coupons.

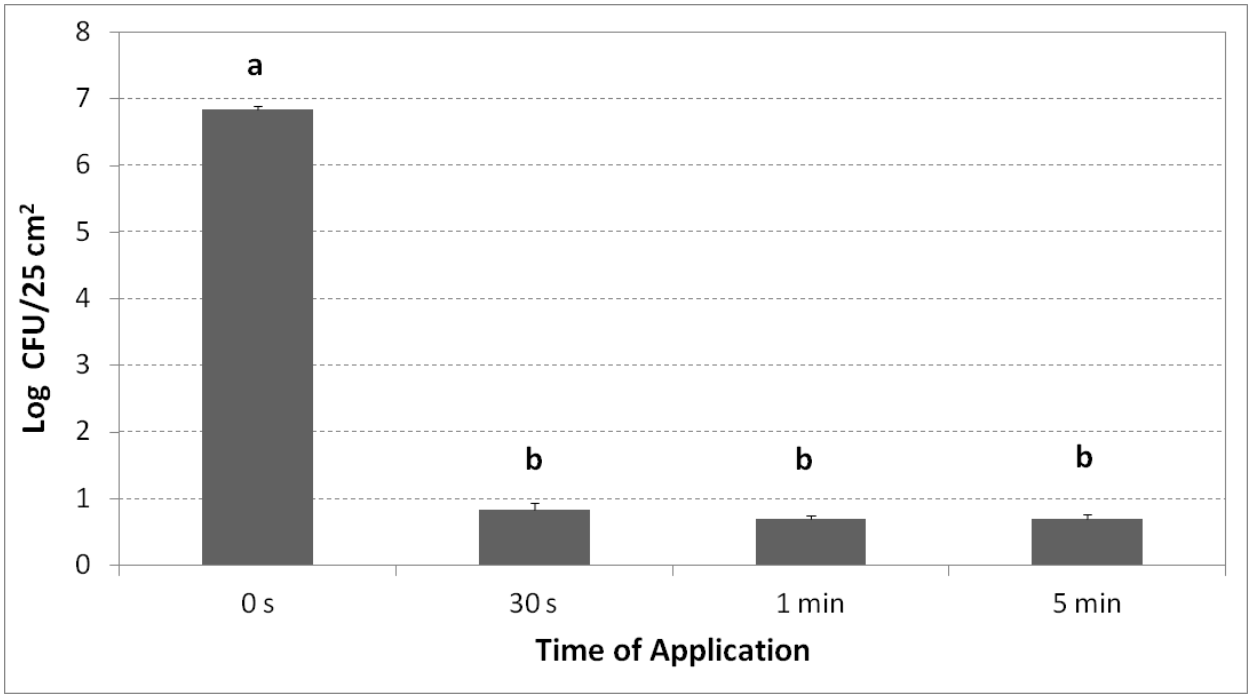

Figure 4.1 *Mean populations (n=12) of* Salmonella *on stainless steel coupons and belting material before and after sanitation exposures using IPAQuat-CO_2 sanitizing system*
[a b] Superscripts indicate differences (p<0.05)

Table 4.1 *Mean populations (n=9) of* Salmonella *serotypes on stainless steel or belting material following exposure to IPAQuat-CO_2 sanitizing system*

		Mean (SE) populations (log CFU/25 cm^2) for exposure time[1]			
Coupon material	Condition	0 s	30 s	1 min	5 min
Stainless steel	Clean	6.93 (0.1) Aa	0.75 (0.2) Bb	<0.70 (0.1) Ab	<0.70 (0.1) Ab
Stainless steel	Soiled	6.72 (0.1) Ba	<0.70 (0.2) Bb	<0.70 (0.1) Ab	<0.70 (0.1) Ab
Belting material	Clean	6.99 (0.1) Aa	1.31 (0.2) Ab	0.72 (0.1) Ac	<0.70 (0.1) Ac
Belting material	Soiled	6.71 (0.1) Ba	<0.70 (0.2) Bb	<0.70 (0.1) Ab	<0.70 (0.1) Ab

[1] Limit of detection 0.70. SE= standard error. AB Means with different capital letters in the same column are different (p <0.05). abc Means with different lowercase letters in the same row are different (p< 0.05).

Discussion

Previous studies have indicated that IPAQuat formula was effective in reducing microbial populations when applied to inoculated bench tops (Jury and others 2010) or added to inoculated almond dust (Du and others 2010). The results of this study confirm findings (Du and others 2010; Jury and other 2010) indicating that IPAQuat formula was effective in reducing microbial populations.

A reported 7 log CFU/g reduction was observed when IPAQuat formula was added to almond dust samples that were mixed with broth cultures of *Salmonella* (Du and others 2010). Sanitizer efficacy studies are often conducted using planktonic cells in broth cultures, but it cannot be assumed that adherent cells will react the same way. False assumptions could be made, especially if cells have an intact glycocalyx (Mosteller and Bishop 1993). These studies were conducted using adherent cells which were attached to stainless steel or conveyor belting materials typically found in dry-processing environments.

While approximately 5 log reductions were observed with a 30 s treatment using IPAQuat-CO_2 sanitation system, low levels of *Salmonella* were enumerated from swabbed surfaces after this treatment**.** Because salmonellosis has been associated with low levels of *Salmonella* in low-moisture products (< 1 CFU/gm) (GMA 2009), low levels of surviving *Salmonella* organisms would not be acceptable. A sanitation treatment of at least 1 min using IPAQuat-CO_2 sanitizing system resulted in 6 log reductions of *Salmonella*. Most swabs results from surfaces treated for 1 min were below the limit of detection (0.70). All swab results from surfaces treated for 5 min were below the limit of detection (0.70). Furthermore, under the conditions of this study, dry-sweeping was not effective in eliminating *Salmonella* from test materials. Dry-sweeping, however, would be a necessary step to remove organic debris before sanitation treatments.

Because salmonellae have been reported to survive in dry environments for long periods and survive on stainless steel for several days (Iibuchi and others 2010; Kusumaningrum and others 2003; Podolak and others 2010), effective cleaning and sanitation is an important step to ensure safe food production and eliminate the potential for products to become contaminated from the processing environment. The IPAQuat-CO_2 sanitizing systems could, therefore, have sanitation applications in dry-processing environments or facilities that process low-moisture

products. Because water is not introduced into the processing environment, risk of spreading *Salmonella* in the processing facility and onto food products is reduced.

Chapter 5 - Summary and Implications

Effective cleaning and sanitation operations directly impact the production of safe foods. Dry-processing environments are particularly challenging to clean and sanitize because water introduced into systems not designed for wet cleaning can favor growth and establishment of pathogenic microorganisms such as *Salmonella*.

Salmonellosis can arise from ingesting very low numbers of *Salmonella* and affects approximately 40,000 individuals annually in the U.S. *Salmonella* outbreaks have been linked to many foods including dry food ingredients and low-moisture products. Control of *Salmonella* is challenging because of its ubiquitous nature. The presence of *Salmonella* in low-moisture products may be attributed to a number of sources including, processing with contaminated (raw) ingredients, and not supplying an adequate heat treatment or from post-thermal, post-processing contamination. Multiple outbreaks of salmonellosis have been linked to cross-contamination in the processing environment. While inhibition of growth has been reported for a_w values below 0.94, *Salmonella* can persist for many years in low-moisture products.

Control of *Salmonella* in dry-processing environments can be challenging if cleaning methods are limited, not effective in the removal of the pathogen or if the cleaning methods employed contribute to cross-contamination. Isopropyl alcohol and quaternary ammonium compounds are widely used for their antimicrobial properties. The isopropyl alcohol quaternary ammonium formula (IPAQuat) containing 58.6% isopropyl alcohol and a blend of quaternary ammonium compounds has been tested and found to be effective against a variety of organisms. The data in this study is consistent with findings (Du and others 2010; Jury and others 2010) indicating that IPAQuat formula was effective in reducing bacterial populations. In this study, IPAQuat formula delivered using CO_2 sanitizing system was effective in reducing populations of *Enterococcus faecium* and *Salmonella* inoculated onto materials typically found in dry-processing environments.

Preliminary results showed that IPAQuat-CO_2 sanitizing system was effective in reducing approximately 3.0 logs of *E. faecium* and *Salmonella* from clean and soiled surfaces after 1 min exposure but initial inoculums had to be at least 10 log CFU/ml to demonstrate a minimum 5 log reduction of organisms after sanitation. In the main study, approximately 7 log

CFU/25 cm^2 were recovered before sanitation treatments, and mean *Salmonella* populations of 0.83, < 0.7, and < 0.7 log CFU/25 cm^2 were recovered after 30 s, 1 and 5 min treatments, respectively. Treatments using IPAQuat-CO_2 sanitation system with 30 s sanitizer exposures resulted in 5.7 log CFU/25 cm^2 reductions whereas, greater than 6.2 log CFU/25 cm^2 reductions were observed for sanitizer exposure times of 1 and 5 min. Therefore, IPAQuat-CO_2 sanitation system reduced over 6 logs CFU/25 cm^2 of *Salmonella* when applied to surfaces for 1 min or more. Because IPAQuat-CO_2 sanitizing system can be sprayed into the processing environment without adding moisture, the system is expected to have sanitation applications in dry-processing, low-moisture environments where control can be challenging.

Suggested future research could focus on the efficacy of IPAQuat-CO_2 sanitation systems against *Salmonella* biofilms in dry-processing environments. The system could also be evaluated for efficacy in reducing other organisms, such as, *Listeria monocytogenes* in the processing environment.

Chapter 6 - References

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Appendix A - Pictorial of Materials and Methods

Figure A.1 *Butterfield's Buffer, letheen broth tubes, L-shaped cell spreaders and cell scrapers*

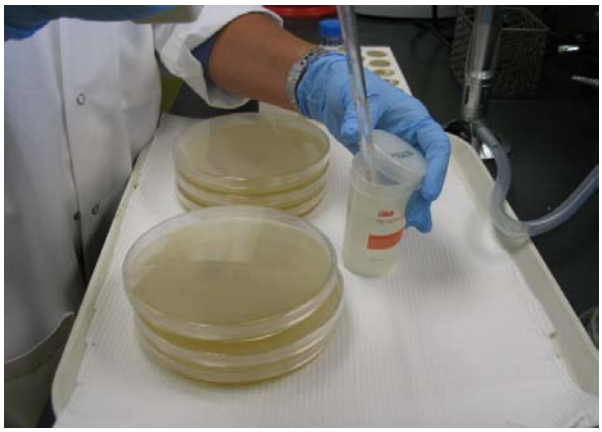

Figure A.2 *Pipetting Butterfield's buffer onto plates to loosen cells*

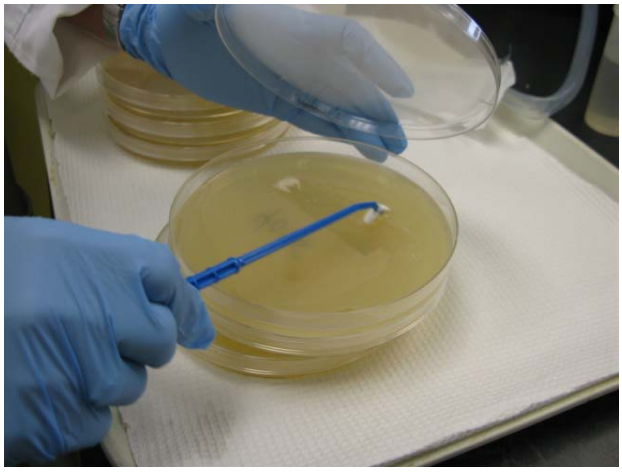

Figure A.3 *Loosening cells with cell scraper*

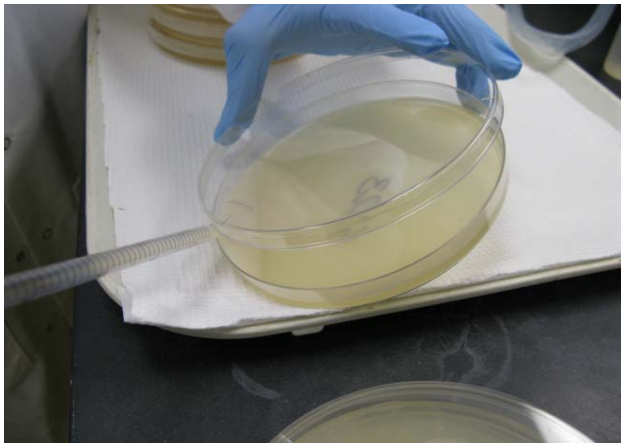

Figure A.4 *Collecting or harvesting cells*

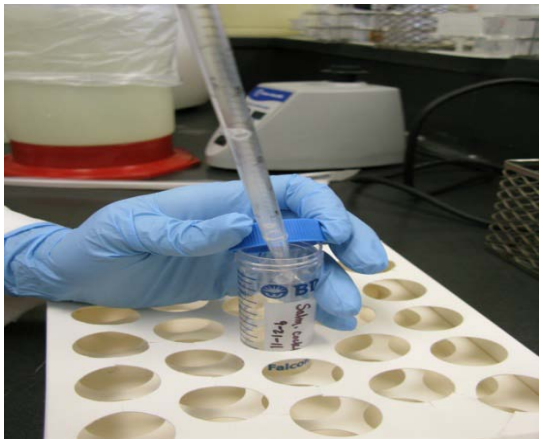

Figure A.5 *Pooling collected cells into plastic conical 50 ml centrifuge tube*

Figure A.6 *Vortexing pooled cell.*

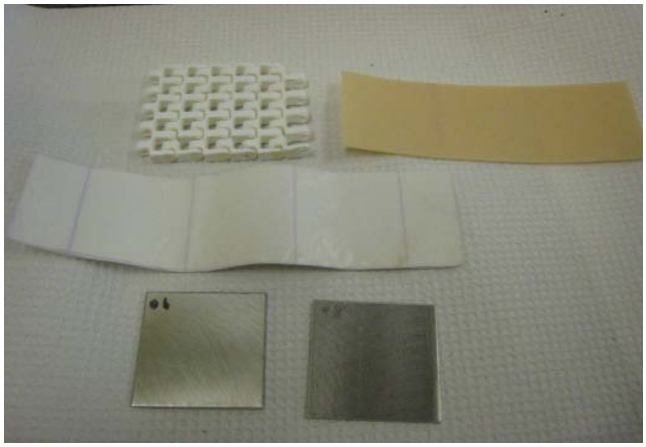

Figure A.7 *Stainless coupons and belting used in preliminary experiment protocols*

Figure A.8 *Repeat pipettor and tips*

Figure A.9 *Inoculating surfaces to prepare for IPA-Quat-CO_2 treatment*

Figure A.10 *Bottles of D2 formula*

Figure A.11 *BioSpray unit used to apply IPA-Quat-CO_2 treatment*

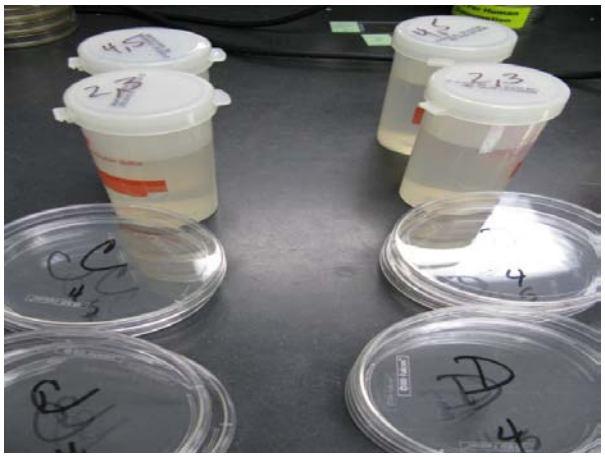

Figure A.12 *Preparing for recovery for untreated surfaces*

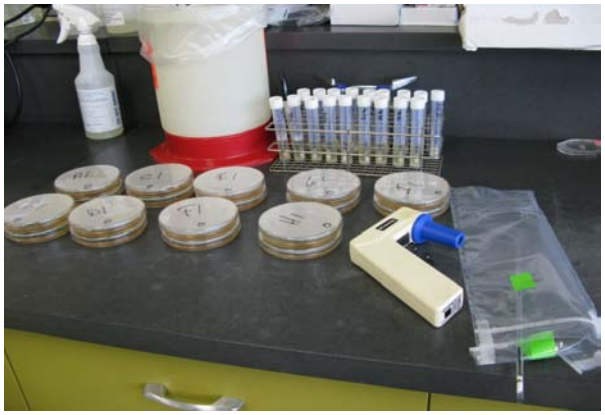

Figure A.13 *Recovery and plating*

Figure A.14 *Belting used in main study experiments*

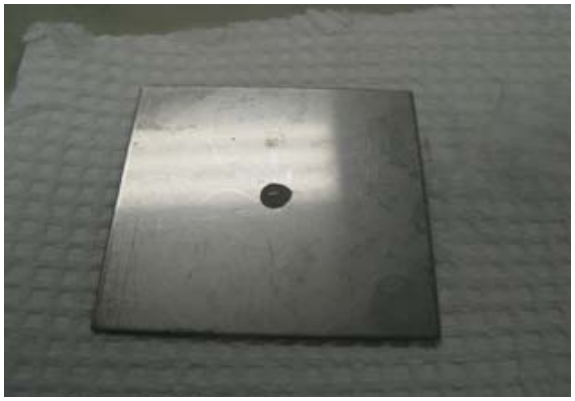

Figure A.15 *Center of 5 × 5 cm coupons were inoculated*

Figure A.16 *Dried inoculum*

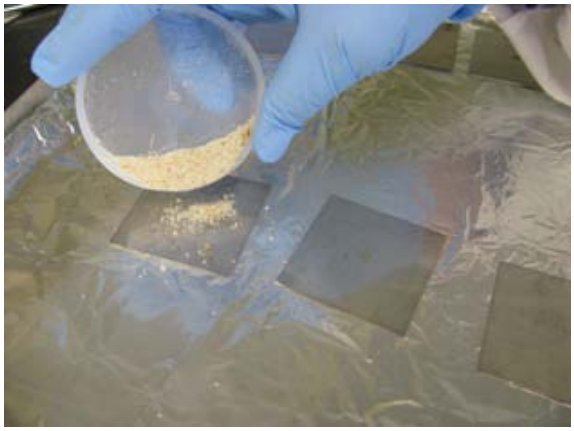

Figure A.17 *Applying soil to coupons*

Figure A.18 *Soiled stainless coupon*

Figure A.19 *BioSpray unit used in main study experiments*

Appendix B - Main Study SAS Code

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1  SS-clean  6  0  6.93
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1  SS-clean  8  0  6.93
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4  SS-clean  3  0  6.96
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5  SS-clean  3  0  6.77
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6  SS-clean  3  0  6.63
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7  SS-clean  2  0  6.80
7  SS-clean  3  0  6.78
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```

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2 SS-clean 8 0.5 .
2 SS-clean 9 0.5 .
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1 SS-clean 8 1 0.7

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3 B-soiled 2 1 0.7

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3  B-soiled  2  5  0.7
3  B-soiled  3  5  0.7
;
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/*
proc mixed data = one covtest;
class rep material time;
model count = material|time/ddfm = satterth;
random rep;
repeated / group = time;
lsmeans material|time/pdiff;
run;
*/
proc mixed covtest data = one;
class rep material time;
model count = material|time/ddfm = satterth;
random rep rep*material*time;
repeated / group = time;
lsmeans material|time/pdiff;
run;
quit;
```